Transbronchial cryobiopsy and Neutrophil Lymphocyte Ratio - new precision medicine tools and markers in Interstitial Lung Disease

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A thesis submitted to University College London for the degree of Doctor of Philosophy

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Declaration

I, Theresia Auguste Mikolasch, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature:

Date:
Acknowledgements

First and foremost, I would like to thank my supervisor Prof Jo Porter for giving me the opportunity to come and work with her at UCL to set up transbronchial cryobiopsies when I was only armed with enthusiasm and a love of bronchoscopy. Throughout the following years I have only ever experienced unwavering support, encouragement, and kindness. Through her guidance I have grown both personally and professionally and I am forever indebted to Jo for pushing me over the finishing line!

I am also grateful to my second supervisor Prof Rachel Chambers and the wider UCL respiratory research group for an environment that truly fosters collaboration and collegial support. I have learnt immensely more than can be reflected in this thesis.

I owe great thanks to my interventional bronchoscopy colleagues Dr Neal Navani and Dr Ricky Thakrar who provided me with the procedural support to carry out our biopsies. Dr Navani was instrumental in initiating the original idea of setting up transbronchial cryo lung biopsies and provided much sense checking and input for various clinical trial protocol drafts. Ricky was my incredible supportive partner in crime in the bronchoscopy suit during most of the procedures and, together with Dr Jagdeep Sahota, covered during my maternity leave to keep the service going.

I am immensely grateful for all the support and enthusiasm Dr Peter Marshall and his colleagues at GSK brought to the MALDI-MS component of this thesis. It all started with me freezing a tomato with my cryoprobe for Peter and continues with our research samples keeping him busy!
I am very thankful to Dr Helen Garthwaite, Dr Jagdeep Sahota and Valerie Holmes for their work on the UCLH ILD database which formed the basis of my NLR work. I am also very grateful to the team at the Nuclear Medicine Department at UCLH under Prof Ashley Groves who provided data for the verification cohort. I further extend my thanks to my colleagues Dr Pete George, Dr Michael Gibbons, Dr Shaney Barratt and Prof Bibek Gooptu who kindly shared their patient cohort data for the NLR work.

I also want to thank the UCLH charity Breathing Matters which kick-started this project and its incredible administrator Donna Basire who was always at hand to help with big or small problems of organisation.

There have been too many other friends and colleagues in our UCL Respiratory family to mention here but the inner (tea) circle know who they are – I could not have wished for a better group of people to help, support, and make me laugh through all the ups and downs of research.

I am blessed with an incredibly supportive husband, Andi, who I met, married, and had my two wonderful sons, Alex (Schlomo) and Max, with during my time at UCL. I could not have done this without him!
Abstract

The interstitial lung diseases (ILDs) are a group of over 200 disease that may lead to progressive fibrosis and respiratory failure. ILDs are heterogenous, with varying amounts of inflammation and fibrosis, and differ in response to therapy and outcome. The most severe fibrotic (f) ILD, idiopathic pulmonary fibrosis (IPF), has a median survival of just three years. Progressive fILD may respond to antifibrotic treatments which slow down, but do not reverse, fibrosis albeit often with significant side effects. Better treatments or delivery of treatments are needed.

Diagnosis of ILD relies on clinical history, imaging and, in some cases lung biopsy, with associated risks. Better diagnostic and prognostic biomarkers in ILD are urgently needed.

This thesis examines the approach to diagnosis, prognostication, and treatment in fILDs, and, in particular IPF. It begins with the finding that Neutrophil Lymphocyte Ratio (NLR), derived from a simple, widely available blood test, is a prognostic biomarker in IPF. The role of lung biopsy in the diagnostic pathway is considered and the use of a relatively new minimally invasive technique of transbronchial cryo lung biopsy (TBCB) as an alternative to surgical lung biopsy (SLB) is described. The value of TBCB to obtain lung tissue for research is demonstrated with evaluation of the distribution of inhaled ipratropium in fILD. Using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) on samples of lung taken using TBCB, it was demonstrated that inhaled medication was able to reach the fibrotic lung, presenting a new approach to drug delivery in fILD. Further discussion focusses on the current role of SLB in the diagnostic pathway in ILD, the presentation of a systematic literature review, and a discussion of future trials to assess the potential benefits of a wider use of TBCB.
Impact Statement

Fibrosing interstitial lung diseases (fILD), especially the most common form Idiopathic Pulmonary Fibrosis (IPF), often have a devastating impact on afflicted patients with the median survival at diagnosis of IPF lower than that of many cancer sufferers. Accurate diagnosis of the underlying fILD is important to lead to correct treatment decisions and inform prognosis. Individual prognosis at the time of diagnosis is hampered by the lack of relevant biomarkers. Available treatment in the form of antifibrotics for IPF and often immunosuppression for other fILD comes with significant side effects in part due to them being administered systemically.

This thesis tackles several of these current clinch points in ILD. The NLR work demonstrates the use of a widely performed, cheap and easy blood tests to help predict mortality outcomes at point of diagnosis which could lead to closer monitoring or earlier initiation of treatment for those at increased mortality risk. It could be further evaluated as a longitudinal biomarker and whether it responds to treatment or could act as biomarker in the context of acute exacerbations.

The establishment of transbronchial cryo-biopsies (TBCB) at UCLH as the first UK centre, opened an alternative diagnostic pathway to the costly and at times harmful surgical lung biopsy. Whilst the technique was pioneered in Germany it had not been established within the NHS prior to this and important differences in terms of patient management and sedation practices form barriers in the UK absent in continental Europe. The UCLH cryobiopsy facility therefore formed an important UK pilot highlighting potential difficulties to other centres considering the establishment of their own service.
The proof-of-concept study combining TBCB with MALDI-MS to demonstrate drug deposition of inhaled ipratropium in fibrotic lung was the first time TBCB was used as a research tool. Its findings open the door to potentially deliver therapeutic compounds directly to the fibrosed lung, therefore potentially avoiding or dampening down systemic side effects caused by oral administration of antifibrotics. These findings could be relevant to other lung diseases.

Using MALDI-MS allows un-altered compound to be detected and spatially related to changes within the lung parenchyma that would not be possible with other drug deposition assessment tools such as radio-labelled imaging studies. Furthermore, it can provide additional information on proteins and other organic compounds in the analysed specimen which potentially could be used to serially assess drug response.
Dedication

I dedicate this thesis to my grandfather who inspired me to never stop learning and exploring. I hope I made you proud Opapa.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
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<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>BAL</td>
<td>Broncho-alveolar lavage</td>
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<tr>
<td>BTS</td>
<td>British Thoracic Society</td>
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<td>CBC</td>
<td>Complete Blood Count</td>
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<tr>
<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
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<tr>
<td>CRF</td>
<td>Case Report Form</td>
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<tr>
<td>CRP</td>
<td>C-reactive Protein</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>CTD-ILD</td>
<td>Connective Tissue Disease – Interstitial Lung Disease</td>
</tr>
<tr>
<td>CTIMP</td>
<td>Clinical Trial of an Investigational Medicinal Product</td>
</tr>
<tr>
<td>DRE</td>
<td>Disease Related Events</td>
</tr>
<tr>
<td>DPLD</td>
<td>Diffuse Parenchymal Lung Disease</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ECM</td>
<td>Extra-cellular Matrix</td>
</tr>
<tr>
<td>eCRF</td>
<td>Electronic Case Report Form</td>
</tr>
<tr>
<td>FEV1</td>
<td>Forced Expiratory Volume in 1 second</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
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<tr>
<td>GAP score</td>
<td>Gender, age, physiology score</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
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<tr>
<td>GSK</td>
<td>GlaxoSmithKline</td>
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<td>H</td>
<td>hours</td>
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<tr>
<td>HCRW</td>
<td>Health and Care Research Wales</td>
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<td>HP</td>
<td>Hypersensitivity Pneumonitis</td>
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<tr>
<td>HRA</td>
<td>Health Research Authority</td>
</tr>
<tr>
<td>HRCT</td>
<td>High Resolution Computed Tomography</td>
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<tr>
<td>ICD</td>
<td>Intercostal Chest Drain</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use</td>
</tr>
<tr>
<td>IIP</td>
<td>Idiopathic Interstitial Pneumonia</td>
</tr>
<tr>
<td>ILD</td>
<td>Interstitial Lung Disease</td>
</tr>
<tr>
<td>IPF</td>
<td>Idiopathic Pulmonary Fibrosis</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>LTOT</td>
<td>Long Term Oxygen Therapy</td>
</tr>
<tr>
<td>MALDI-MS</td>
<td>Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry</td>
</tr>
<tr>
<td>mcg</td>
<td>micrograms</td>
</tr>
<tr>
<td>MDT</td>
<td>Multidisciplinary Team</td>
</tr>
<tr>
<td>MedDRA</td>
<td>Medical Dictionary for Regulatory Activities</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeters</td>
</tr>
<tr>
<td>MSI</td>
<td>Mass Spectrometry Imaging</td>
</tr>
<tr>
<td>NHS</td>
<td>National Health Service</td>
</tr>
<tr>
<td>NLR</td>
<td>Neutrophil-Lymphocyte-Ratio</td>
</tr>
<tr>
<td>NSIP</td>
<td>Non-Specific Interstitial Pneumonia</td>
</tr>
<tr>
<td>OLB</td>
<td>Open Lung Biopsy</td>
</tr>
<tr>
<td>PF-ILD</td>
<td>Progressive fibrosing Interstitial Lung Disease</td>
</tr>
<tr>
<td>PRISMA</td>
<td>Preferred Reporting Items for Systematic Reviews and Meta-Analyses</td>
</tr>
<tr>
<td>PIS</td>
<td>Participant Information Sheet</td>
</tr>
<tr>
<td>RBH</td>
<td>Royal Brompton Hospital</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>RDE</td>
<td>Royal Devon and Exeter Hospital</td>
</tr>
<tr>
<td>REC</td>
<td>Research Ethics Committee</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver Operating Characteristic</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
</tr>
<tr>
<td>SLB</td>
<td>Surgical Lung Biopsy</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>SPC</td>
<td>Summary of Product Characteristics</td>
</tr>
<tr>
<td>SRM</td>
<td>Study Reference Manual</td>
</tr>
<tr>
<td>TBCB</td>
<td>Transbronchial Cryobiopsy</td>
</tr>
<tr>
<td>TLCO</td>
<td>Transfusion Capacity for Carbon Monoxide</td>
</tr>
<tr>
<td>UCL</td>
<td>University College London</td>
</tr>
<tr>
<td>UCLH</td>
<td>University College London Hospitals</td>
</tr>
<tr>
<td>UIP</td>
<td>Usual Interstitial Pneumonia</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>VATS</td>
<td>Video-Assisted Thorascopic Surgical procedure</td>
</tr>
</tbody>
</table>
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Chapter 1 Introduction
1.1 Interstitial Lung Diseases

Interstitial lung disease (ILD) is an umbrella term for a group of more than 200 different lung disease and there is considerable variation in underlying causes, treatment options and prognosis.

Broadly speaking ILDs can be sub-divided into known causes through exposure such as drug reactions, occupational exposure (e.g. asbestosis), allergen exposure (hypersensitivity pneumonitis) or radiation damage; known causes through association with systemic disease such as ILDs associated with connective tissue diseases (ILD-CTD), sarcoidosis or ILD associated with Inflammatory Bowel disease. Other ILDs are familial or genetic such as familial Idiopathic Pulmonary Fibrosis while others do not fit into clear categories such as lymphangioleiomyomatosis (LAM). Probably the most challenging group are the so call Idiopathic Interstitial Pneumonias (IIPs) which have no known cause or association and are often difficult to differentiate and treat. Figure 1.1 represents a schematic break down of the main category.
Figure 1.1: Classification of ILDs (Adapted from Zibrak et al) (1)
There is considerable variation in the prognosis and treatment options of different IIPs. Idiopathic Pulmonary Fibrosis (IPF) is the most common with a median survival of 3 years causing 5000 deaths/yr in the UK. The incidence increases with age and IPF is more common in men. A UK study reported an increase of IPF by 11% annually between 1991 and 2003 which was felt not to be attributable to the ageing of the population or increased diagnosis. It therefore appears to be a growing problem (2).

The incidence of IIPs and IPF, in particular, will continue to rise in the future due to the ageing population making it highly relevant to the needs of the NHS, present and future. IPF and other ILDs, especially Non-Specific Interstitial Pneumonia (NSIP), are often difficult to distinguish without histological analysis of lung tissue gained through SLB (3, 4, 5, 6, 7). There is a paucity of management options for these devastating diseases, but treatments are slowly emerging and accurate diagnosis is increasingly important. Mounting evidence suggests that immunosuppression in NSIP and other fibrotic ILD such as hypersensitivity pneumonitis (HP) with agents such as cyclophosphamide(8) and rituximab(9) is beneficial whereas aggressive immunosuppression in IPF increases mortality(10); the anti-fibrotic agents pirfenidone and nintedanib are the only licensed pharmacological treatment for IPF, but come with frequent side effects, and a high annual cost which has led to prescribing restrictions in England reserving them currently only for patients with a diagnosis of IPF, making accurate patient selection essential(11).
Since various forms of ILD such as IPF, non-IPF forms of IIP, CTD-ILD, and HP can have similar clinical presentations, patients with suspected ILD must undergo an evaluation that adequately establishes a confident diagnosis of an individual ILD, as treatment and certain management decisions are diagnosis-specific and may vary considerably according to the particular form of ILD that is diagnosed. Early accurate diagnosis leads to early targeted treatment and in turn better outcomes. This is especially important in the progressive fibrosing forms of ILD as any lost lung function cannot be recovered (12).

1.1.1 Diagnostic work up in Interstitial Lung Diseases

Initial diagnostic work up in all newly presenting ILD patients is focused on a thorough clinical history aimed at establishing potential triggers and exposures such as occupational or drug exposure as well as potential allergens animal contacts together with clinical assessment for connective tissue diseases (CTD) and other associated systemic diseases. This is followed by autoantibody and serology blood tests to further investigate potential ILD-CTD or exposure to antigens associated with HP such as Aspergillus or Avian antigens.

Full pulmonary lung function testing is required to assess severity of disease and to be able to monitor progress and treatment response.

Echocardiography is also frequently performed to exclude underlying cardiovascular disease which might be contributing to symptoms of dyspnoea and exhaustion. It is also utilised to assess for pulmonary hypertension which can present as a sequel of chronic pulmonary disease or as part of the underlying disease process as is the case in scleroderma associated ILD.
Radiological Work up

Chest Radiographs

Chest radiographs can show a multitude of abnormalities depending on the underlying sub-form of ILD but can on occasion also be normal. Some of the more specific findings identifiable on chest x-ray (CXR) include interstitial infiltrates associated with calcified pleural plaques pointing towards a diagnosis of asbestosis or bilateral hilar lymphadenopathy making sarcoidosis more likely.

The distribution pattern of interstitial changes can also be helpful in narrowing down the list of differential diagnosis- for example upper lobe predominance is often associated with chronic HP whereas the lower lobes are mostly affected in IPF or NSIP. Asymmetrical distribution can point towards a degree of gastro-oesophageal reflux and aspiration contributing to interstitial damage.

CXRs are rarely sufficient to make a confident diagnosis of a specific ILD but can prompt appropriate further imaging and can be useful in tracking the progression of disease. If previous CXRs are available their review can establish if the interstitial process is chronic or acute.

High Resolution Computed Tomography

High-resolution computed tomography (HRCT) of the thorax is a key component of the diagnostic evaluation in suspected ILD. It is nearly universally obtained in patients with suspected ILD and may be diagnostic removing the need for invasive approaches such as bronchoscopy or surgical lung biopsy (SLB). The quality of HRCT images and their utility in ILD diagnosis depends on the scanning protocol employed. Traditionally images were
obtained at 1 to 2 cm cross-sectional intervals which meant that small focal abnormalities were not visualised and breathing artefacts common. The use of multidetector CT scanners makes it possible to scan the entire thorax in a single breath-hold. These images can be reconstructed to contiguous high-resolution images.

The ATS/ERS consensus statement (13) for the diagnosis of IPF set out criteria for the optimal HRCT technique for evaluation of ILD.

Interpretation of HRCT scans in ILD is based on assessing the extent, specific distribution, and severity of the following findings: parenchymal reticulation, centri-lobular nodules, foci of low attenuation, ground-glass attenuation, traction bronchiectasis, air trapping, architectural distortion, and honeycombing. The 2008 Fleischner Society statement (14) has attempted to clarify and standardise the terminology employed when describing HRCT scan findings in ILD but there remains considerable variability and inter-observer variation in diagnosis and confidence of diagnosis. This in part depends on the setting (district hospitals or tertiary referral centres) as well as complexity of the case (15). Sensitivity and specificity of radiological diagnosis also depends on confidence of diagnosis and the nature of the underlying diagnosis. In a retrospective study of NSIP, IPF and chronic HP cases a confident radiological diagnosis was reached in 53% of HRCT readings (n=132) (16).

A definitive Usual Interstitial Pneumonia (UIP) pattern on HRCT has a specificity of approximately 95% and sensitivity of approximately 40% for UIP. In contrast, a predominant feature of ground glass opacities (GGOs) gives a
sensitivity of approximately 95% and specificity of approximately 40% for NSIP (17). In recent years evidence has grown that the absence of honeycombing on a scan otherwise displaying features of UIP does not significantly change the likelihood of a corresponding histological UIP diagnosis (18).

Therefore, the most recent ATS/ERS consensus statement has set out diagnostic criteria for a probable UIP pattern which allows diagnosis of IPF in the right clinical setting without the need for histopathological confirmation of UIP (19). (Table 1.1)
<table>
<thead>
<tr>
<th>UIP</th>
<th>Probable UIP</th>
<th>Indeterminate for UIP</th>
<th>Alternative Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subpleural and basal predominant; distribution is often heterogeneous*</td>
<td>Subpleural and basal predominant; Distribution is often heterogenous Reticular pattern with peripheral traction bronchiectasis or bronchiolectasis</td>
<td>Subpleural and basal predominant Subtle reticulation; may have mild GGO or distortion (“early UIP pattern”) CT features and/or distribution of lung fibrosis that do not suggest any specific aetiology (“truly indeterminate for UIP”)</td>
<td>Findings suggestive of another diagnosis</td>
</tr>
<tr>
<td>Honeycombing with or without peripheral traction bronchiectasis or bronchiolectasis†</td>
<td>May have mild GGO</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1 HRCT Criteria for UIP Pattern - Adapted from ATS/ERS 2018 Guideline (8)

Therefore, HRCT appearances consistent with an UIP pattern make the diagnosis of IPF highly likely and additional interventional diagnostics such as bronchoscopy or SLB are unlikely to change the consensus diagnosis. However, in cases with less clear radiology, especially when the clinical context is not typical, additional histological information might be required to make a diagnosis.
Bronchoscopy in the assessment of ILD

Bronchoalveolar Lavage

Considerable debate and variation in practice surrounds the use of bronchoalveolar lavage (BAL) in the diagnostic work up in ILD. A weak negative recommendation was made against BAL in the majority of patients in the 2011 ATS/ERS IPF diagnostic consensus guidelines but the ensuing controversy led to the ATS publishing guidelines about the role of BAL in ILD diagnosis in 2012 (20) and it has now received a conditional recommendation in the latest guidelines (19). While undoubtedly valuable in excluding infection BAL can only rarely be diagnostic by itself (with notable exceptions, for example, eosinophilic pneumonia); however, it can provide valuable additional information when narrowing down the list of possible differential diagnosis.

The ATS guidelines concede that BAL cellular analysis may be useful in individuals who lack a confident UIP pattern HRCT. 2013 NICE guidelines for the diagnosis and management of suspected IPF state that BAL might be beneficial in the work up and do not directly refer to HRCT appearances (11). When deciding whether to perform BAL the treating physician must take into consideration the degree of uncertainty about the type of ILD, the likelihood that the BAL will provide helpful information, and whether the patient would tolerate the procedure as well as patient’s wishes.

Recognition of a predominantly inflammatory cellular pattern (increased lymphocytes, eosinophils, or neutrophils) in the BAL differential cell profile frequently helps the clinician narrow down the list of potential diagnosis. Table 1.2 sets out some recognised differential cell count patterns.
I. Healthy Non-smokers

<table>
<thead>
<tr>
<th>BAL Different Cell Counts</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar macrophages</td>
<td>&gt;85%</td>
</tr>
<tr>
<td>Lymphocytes (CD4+/CD8+ = 0.9-2.5)</td>
<td>10-15%</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>&lt;3%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Squamous epithelial*/ciliated columnar epithelial cells†</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>

II. Interstitial lung diseases

<table>
<thead>
<tr>
<th>Disorders associated with increase percentage of specific BAL cell types</th>
<th>Lymphocytic cellular pattern</th>
<th>Eosinophilic cellular pattern</th>
<th>Neutrophilic cellular pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;15% lymphocytes</td>
<td>&gt;1% eosinophils</td>
<td>&gt;3% neutrophils</td>
<td></td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>Eosinophilic pneumonias</td>
<td>Collagen vascular diseases</td>
<td></td>
</tr>
<tr>
<td>Nonspecific interstitial pneumonia (NSIP)</td>
<td>Drug-induced pneumonitis</td>
<td>Idiopathic pulmonary fibrosis</td>
<td></td>
</tr>
<tr>
<td>Hypersensitivity pneumonitis</td>
<td>Bone marrow transplant</td>
<td>Aspiration pneumonia</td>
<td></td>
</tr>
<tr>
<td>Drug-induced pneumonitis</td>
<td>Asthma, bronchitis</td>
<td>Infection: bacterial, fungal</td>
<td></td>
</tr>
<tr>
<td>Collages vascular diseases</td>
<td>Churg-Strauss syndrome</td>
<td>Bronchitis</td>
<td></td>
</tr>
<tr>
<td>Radiation pneumonitis</td>
<td>Allergic bronchopulmonary aspergillosis</td>
<td>Asbestosis</td>
<td></td>
</tr>
<tr>
<td>Cryptogenic organizing pneumonia (COP)</td>
<td>Bacterial, fungal, helminthic, <em>Pneumocystis</em> infection</td>
<td>Acute respiratory distress syndrome (ARDS)</td>
<td></td>
</tr>
<tr>
<td>Lymphoproliferative disorders</td>
<td>Hodgkin’s disease</td>
<td>Diffuse alveolar damage (DAD)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.2 Summary of BAL cellular patterns in different ILDs (Adapted from Meyer et al) (20)
An interesting study published in 2009 detected a BAL lymphocytosis of >30% in 6 out of 74 patients with definite UIP features on HRCT and in all six cases an alternative diagnosis to IPF was made. Therefore the importance of routine bronchoscopic assessment in suspected IPF remains unclear and recent NICE guidance has included research recommendations into the value of bronchoalveolar lavage (BAL) in the diagnostic pathway(11). BAL is generally well tolerated with an excellent safety profile though case reports exit of IPF exacerbations following routine bronchoscopy(21).

**Endobronchial Ultrasound and Transbronchial Needle Aspiration**

Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) is a well-established bronchoscopic technique to sample enlarged mediastinal lymph nodes. Lymph node stations accessible by this technique are the paratracheal, subcarinal, hilar and interlobar lymph node stations (stations 2,4,7,10,11). Several fibrosing ILDs can present with a degree of mild mediastinal enlargement which is usually reactive and not actively investigated. The main role of EBUS-TBNA in the ILD diagnostic pathway is to assess larger mediastinal lymphadenopathy in suspected sarcoidosis and to exclude alternative pathology such as tuberculosis, lymphoma or other underlying malignancy.

It may be performed under conscious sedation and again has an excellent safety profile.
Transbronchial Biopsy with forceps

Transbronchial biopsy (TBBx) utilising standard bronchoscopy forceps is a minimal invasive technique for obtaining lung tissue for histological diagnosis in many pulmonary disorders. However TBB does not always provide adequate or histologically acceptable lung tissue to set a final diagnosis of heterogeneous lung diseases as the biopsies are small, and subject to crush artefact and may not be representative in spatially heterogeneous disease (22, 23). It can be helpful in diagnosis of ILDs associated with distinctive histopathological features such as granulomas in sarcoidosis that can be diagnosed on small specimens and do not depend on recognising specific architectural features as in IPF. Berbescu et al showed that the contribution of TBB in diagnosis of IPF is only 32% (24). Worryingly, small sample size and crush artefact in lung tissue acquired during TBB may mislead the pathologist and potentially lead to incorrect diagnoses in the case of IIPs such as IPF and NSIP (25).

The total number of biopsies taken for optimal diagnostic yield is reported to be 4-10 (26). The most common complications are pneumothorax (2-6%) and significant bleeding (2-9%) post procedure. The pneumothorax rate may be reduced by using fluoroscopy during the procedure.
Transbronchial Cryobiopsy

Transbronchial cryobiopsy (TBCB) was first described in 2009(27). Since then, it has been shown be a safe, and effective minimal invasive diagnostic tool for the histological diagnosis of ILD, with a mean diagnostic yield of 71.9% (SD14.4, 95% CI 66.9-76.9) (See Chapter 6). The advantage of TBCB over conventional forceps TBB lies in the larger specimen size and lack of crush or bleeding artefacts distorting the tissue architecture.

An up to date literature review on TBCB is discussed in chapter 6 of this thesis.

Surgical Lung Biopsy

Surgical lung biopsies (SLB) are the current gold standard for obtaining histological material in the diagnosis of IIPs. It is usually performed via the less invasive Video-Assisted Thoracoscopic Surgical (VATS) approach rather than an open thoracotomy biopsy. Following the 2011 ATS/ERS consensus statement about two thirds of cases of IPF can be diagnosed based on typical radiological findings of UIP and clinical picture. Therefore, around a third of patients with suspected IPF would require SLB to confirm or refute the diagnosis. With the recent change in guidelines the proportion of patients requiring SLB has diminished somewhat. We estimate from two surveys that only 7.5%-12% of suspected IPF patients undergo SLB in the UK (28, 29). This may reflect the reluctance of clinicians to refer patients for a procedure associated with significant mortality and morbidity.
The average hospital stay associated with VATS biopsy is 2-5 days (30). Mortality has been reported as 3-4% and overall complication rate up to 16% (31). 57% of patients report pain at the incision site 6-12 months after surgery (32). Other common complications include persistent air-leak; exacerbations of underlying ILD due to mechanical stress of single lung ventilation; bleeding and delayed wound healing.

A 2014 paper from Scotland reported a case series of ILD patients undergoing VATS lung biopsy reported a 1.5% 30-day mortality and complications in 28.8% with an average hospital stay of 3.53 days. A definite pathological diagnosis was reached in 74.2% (other studies range from 34%-100%) (30). This can be regarded as an up-to-date benchmark for VATS biopsies in the UK.

In 2012, 910 SLB were performed in the UK(33) for suspected ILD at an estimated cost of £3 million; Considering an average hospital stay of about 3.5 days this amounts to 8.7 patient years (PY) spent in hospital. If guidelines were to be followed and 1/3 of cases were biopsied costs would increase to £5million/yr. and 16 PY/yr. would be spent in hospital; with the projected rise in IIP cases the strain on the NHS will further increase.

A systematic literature review of SLB in ILD is reported in chapter 4 of this thesis.

**Multidisciplinary Team**

No single diagnostic test can be considered to provide a definite, confident diagnosis in almost all ILDs. Therefore, a consensus diagnosis reached by a Multidisciplinary Team (MDT) with expertise in ILD is now considered the gold
standard. The MDT can integrate all available data at several stages of the diagnostic work up. This does not only improve inter-observer agreement and diagnostic confidence (34) but may also prevent unnecessary invasive biopsies and identify patients in whom a biopsy may effectively contribute to the diagnosis. Current NICE guidelines recommend that IPF should only be diagnosed by MDT consensus (11). It also recommends a minimum MDT composition of one consultant respiratory physician, one consultant radiologist, an ILD specialist nurse and an MDT co-ordinator all of whom should have expertise in ILD. When invasive diagnostics are considered a consultant histopathologist and, if appropriate, a consultant thoracic surgeon should also form part of the MDT. There is some evidence that involving more than one clinician of the same specialty in a MDT increases inter-observer agreement between specialties (35).

Figure 1.2 provides an overview of the diagnostic decision tree in the work up for a potential IPF diagnosis.
Figure 1.2: ATS/ERS Diagnostic algorithm for idiopathic pulmonary fibrosis (IPF) (adapted from Raghu et al)

(19)

BAL- bronchoalveolar lavage, MDD- multidisciplinary discussion, HRCT- High Resolution Computed Tomography.

UIP – Usual Interstitial Pneumonia
### 1.2 Prognostic markers in IPF

Prognosis for different ILDs can vary wildly depending on the underlying disease process and can be especially challenging in IPF which can have a varied disease course, from rapid progression to relative indolence. While a multitude of prognostic biomarkers in ILD have been evaluated and shown to correlate with disease progression, no simple test is used in every day clinical practice that can predict outcome at the time of diagnosis. In IPF specifically markers of epithelial cell dysfunction and extra-cellular matrix (ECM) remodelling such as MMP-7 (36) and KL-6 (37) have been associated with accelerated clinical decline when elevated in serum. The PROFILE study (Prospective Observation of Fibrosis in the Lung Clinical Endpoints) identified six collagen derived markers of ECM turnover in serum that if elevated correlated with a faster progressing phenotype of IPF (38). Genetics polymorphism of MUC5B and TOLLIP have also been shown to impact on likelihood of progression (39). While the discovery and investigation of these biomarkers have revealed important mechanistic of the underlying pathological processes they are not fit for day-to-day clinical practice.

Widening the definition of biomarker to include any naturally occurring characteristic or measurement brings us to the tools used in clinical practice to predict outcomes in IPF. These are composite risk models such as the GAP index which is compiled from gender, age, and physiological markers (DLCO and FVC) and used to predict average risk of mortality (40). The Composite Physiologic Index is derived from physiological markers (DLCO, FVC and FEV1) and disease extent on CT imaging and has been shown to predict
mortality more accurately than lung function alone by correcting for emphysema as a confounding factor (41).

Predicting likely disease course early has important implications for follow-up intervals, timing of initiation of disease modifying therapy such as antifibrotics, referral for lung transplant evaluation. The ideal predictive biomarker should be quick, cheap, easy to access and reliable.
1.3 AIMS OF THIS THESIS

This thesis aims to

- Establish TBCB as a research tool and to assess its suitability as an alternative to SLB in gaining histopathology.
- Deliver a proof-of-concept clinical trial combining TBCB and MALD-MS imaging to proof drug deposition in fibrotic human lung.
- To identify the ideal NLR cut off point to predict mortality in IPF and validate in a larger external validation cohort
1.4 CONTRIBUTIONS TO THE WORK REPORTED IN THIS THESIS

The Neutrophil Lymphocyte Ratio study was designed and executed by me. I wrote the study protocol and ethics applications, arranged sponsorship and adoption by the UCL R&D office. I organised relevant contracts and data sharing agreements for the additional sites. I performed data collection with the help of my colleague Dr Jagdeep Sahota and the Nuclear Medicine research team at the UCLH site. The data from the other five cohorts were collected by local collaborators at the respective sites. I performed all data cleaning and statistical analysis and modelling.

I set up the transbronchial cryobiopsy service at UCLH. I assessed all patients personally (par 3 patients who were biopsied during my absence on maternity leave) and performed most procedures with the help of Dr Ricky Thakrar and Dr Neal Navani.

For the GSK drug deposition trial, I had key involvement in developing the protocol and responsibility for delivering the protocol, study documents, and ensuring the study was compliant with information and data governance regulations. I organised relevant contracts and data sharing agreements. I was solely responsible for recruitment, study participant assessment, carrying out the study procedure and collecting research samples and relevant data. I was responsible for carrying out most of the data collection, supported by our research nurses Therese Bidder and Geeta Vekaria. I liaised with the sponsor at site visits.
I had some involvement in the sample processing for MALDI MS analysis, but most of the sample processing and data analysis was carried out by Dr Peter Marshall and his team at GSK, Stevenage.

With regards to the unfulfilled clinical trials of LUNG COOL and TREATS. I developed and wrote the protocol and ethics applications for both with guidance from my senior colleagues (especially Dr Neal Navani) and the Clinical Trial Unit Team. I wrote the Health Technology Assessment Funding application for the LUNG COOL trial and presented the study at the local ethics committee.

I have had guidance and support through all this work by my supervisor Prof Jo Porter and the wider team at UCL/UCLH. They are further acknowledged in the acknowledgment section.
Chapter 2

Exploring the current gold standard of histological diagnosis

–

Surgical lung biopsy in the diagnosis of ILD

–

A systematic Literature Review
2.1 introduction

As outlined in Chapter 1, surgical lung biopsy (SLB) remains the recommended gold standard for obtaining lung tissue for histopathology in ILDs in which the diagnosis cannot be reached confidently after MDT discussion taking into consideration clinical, radiological, and bronchoscopic assessments. It is usually performed via the less invasive Video-Assisted Thoracoscopic Surgical (VATS) approach rather than an open thoracotomy biopsy. Despite increasing confidence in radiological assessments, SLB continues to be featured and recommended in the most recent diagnostic guidelines including the 2020 ATS/JRS/ALAT diagnosis of hypersensitivity pneumonitis (42) and the 2018 ATS/ERS/JRS/ALAT IPF (19) guidelines, though the suspected IPF patient group in whom it is strongly recommended has reduced with the introduction of the “probable UIP” pattern compared with the previous re-iteration of the guideline in 2011(13).

Anecdotally, the use of SLB in the diagnosis of ILDs has been falling out of favour over the last few years, especially in the UK. This is in part due to greater confidence in other diagnostic assessments (mainly HRCT) and a growing awareness of the associated morbidity and mortality, which is increasingly seen as unacceptable for a diagnostic procedure.

To methodically quantify the risks and burden (mortality and morbidity as well as length of stay) and utility (pathological and diagnostic yield, change in treatment) associated with SLB, we conducted a systematic literature review in 2015 following the PRISMA (43) approach with a pre-specified protocol (see appendix 6), two reviewers, and quality assessment of papers included.
results of this systematic review are presented in Part A of this chapter. In 2020 this literature search was updated using the same search terms and inclusion/exclusion criteria as in the original review, however this was only performed by one reviewer. Its findings have been summarised narratively in Part B.

The differing results from these two searches are then compared and discussed in the discussion of this chapter.
SURGICAL LUNG BIOPSY IN THE DIAGNOSIS OF ILD – SYSTEMATIC LITERATURE REVIEW

Primary Reviewer: Dr Theresia Mikolasch, University College London, London

Secondary Reviewer: Dr Adam Marshall, Royal Infirmary of Edinburgh, Edinburgh

Abstract

Methods We performed a systematic review across Pubmed and Embase databases for studies reporting the use of Surgical Lung Biopsies (SLB) in the diagnosis of adults with Interstitial Lung Disease (ILD). Randomised controlled trials, case control studies and case series with more than 20 subjects were included, restricted to papers published from 2000 onwards taking into consideration changes in surgical techniques and diagnostic criteria. Primary outcomes were 90-day mortality and complications while secondary were diagnostic yield, mean length of stay and change of treatment following biopsy.

Results 24 studies were included reporting on the use of SLB in 2600 patients. The overall quality of the reports was moderate to poor with mainly retrospective case series available. Mean mortality was 4.9% (CI 90% -0.04 - 0.14) with a wide range of 0- 22.4%. Complication rates were reported in 19 of the studies. Mean overall complication rate was 19.4% (CI -0.05- 0.48) with a range from 7.1% to 65.7%. Mean length of stay adjusted for patient
numbers was 5.4 days and diagnostic yield for definite pathological diagnosis was 89%. Eight studies recorded treatment change following SLB in a total of 588 patients out of 869. Mean percentage of patients in whom treatment was changed based on the SLB result was 60% (CI 90% 0.35-0.87).

Conclusions High-quality data on the outcomes of SLB in ILD diagnosis are sparse. Comparison between different studies is difficult due to heterogeneous patient populations and differences in outcome reporting. SLB in ILD remains a useful diagnostic tool but carries significant mortality and morbidity. More prospective data and evaluation of surgical risk stratification is required.
Methods

We used a systematic review methodology based on the PRISMA(43) approach and principles. As the authors were aware that high-quality trials data is lacking in this subject field, we specifically allowed consideration of case series within the summation of the literature. The review process followed a pre-specified protocol (see Appendix 6).

Types of Studies

Original research studies and case series published in English evaluating the use of SLB in Interstitial Lung Disease. Minimum of 20 patients enrolled. Studies were included if they recorded mortality and/or complication rates. Exclusions consisted of the following: letters, case reports, editorials and reviews. Studies where the final diagnosis in a significant proportion (>20%) of patients was not ILD (such as infection, malignancy).

Types of Participants

Adults only with evidence of Interstitial Lung Disease or Diffuse Parenchymal Infiltrates on cross sectional imaging

Types of Interventions

Surgical lung biopsy was defined as either open lung biopsy (OLB) or “video-assisted thoracoscopic surgery” (VATS). Studies evaluating non-standard surgical techniques were excluded such as surgery without general anaesthetic or medical thoracoscopy.
Outcomes

Primary Outcomes

- Mortality
- complication rates (including but not limited to prolonged air leak; wound infection; bleeding; pneumothorax; exacerbation of underlying lung disease)

Secondary outcomes

- Length of stay
- Pathological diagnostic yield
- Overall diagnostic yield (taking into consideration clinical and radiological information)
- Treatment changes following SLB.
Search Methods

The optimal search strategy for identifying trials in Embase and PubMed was modified to include MeSH and free-text terms for surgical lung biopsy and searched from 2000 to present. In addition to electronic database scrutiny, we hand-searched textbooks and reference lists of included studies and articles.

PUBMED search accessed 29/07/15.

(((((((("histopathology") OR "video assisted thoracic surgery") OR "video assisted thoracoscopic surgery") OR "VATS") OR "open lung biopsy") OR "surgical biopsy") OR "surgical lung biopsy") AND ((("diffuse parenchymal lung disease*") OR ILD) OR "interstitial lung disease*") OR "parenchymal infiltrate*") OR "pulmonary fibrosis") OR "lung fibrosis") AND ("2000/01/01"[PDat] : "2015/12/31"[PDat])))) AND english[Language] AND (hasabstract[text] AND English[lang])

Embase search:

1. pulmonary fibrosis.mp. or lung fibrosis/
3. *lung fibrosis/ or *interstitial lung disease/ or *interstitial pneumonia/ or diffuse parenchymal lung disease*.mp. or *fibrosing alveolitis/ or *lung parenchyma/
4. *interstitial lung disease/ or ILD.mp. or *lung fibrosis/
5. *lung infiltrate/ or diffuse parenchymal infiltrate*.mp.
6. 1 or 2 or 3 or 4 or 5
7. *lung biopsy/ or surgical lung biopsy.mp.
8. open lung biopsy.mp. or *open lung biopsy/
9. VATS.mp. or *video assisted thoracoscopic surgery/

10. *thorax surgery/ or video-assisted thoracoscopic surgery.mp. or
*thoracoscopy/ or *video assisted thoracoscopic surgery/

11. thoracic surgery.mp. or *thorax surgery/

12. 7 or 8 or 9 or 10 or 11

13. 6 and 12


15. mouse.ab.

16. child.ab.

17. infant*.ab.

18. pediatr*.ab.

19. 13 not 14

20. 19 not 15

21. 20 not 16

22. 21 not 17

23. 22 not 18

24. review.pt.

25. 23 not 24

26. limit 25 to (abstracts and english language and yr="2000 -Current")

27. limit 26 to human
Selection of Studies
All relevant abstracts were assessed by two independent reviewers (TM and AM) utilising EPPI reviewer 4, an online software tool for research synthesis developed and maintained by the EPPI-Centre at the Social Science Research Unit of the UCL Institute of Education, University of London, UK(44). Full papers were obtained, where available, for those deemed potentially eligible, and the two reviewers agreed the final set of review papers. See Figure 2.1 for details.
Electronic database & manual searches (N=1172)

Duplicates excluded (n=210)

Abstract screen & search (n=962)

Did not meet inclusion criteria (n=912)

Full text screen & search (n=50)

Did not meet inclusion criteria (n=26)

Reports for synthesis (n=24)

Figure 2.1 PRISMA flow diagram of evidence synthesis.
Data Extraction and Management

The following data sets on study characteristics, patient characteristics, interventions and outcomes were extracted from publications onto an electronic form (Microsoft Excel 2010, Microsoft Corp, USA).

Study characteristics

- Authors
- Study size
- Retrospective vs prospective
- Location

Patient characteristics

- Age
- Sex
- Lung Function
- Pre-biopsy diagnosis

Intervention

- Surgical approach used (VATS vs OLB)
- Number of biopsies taken
- Biopsy sites
Outcomes

- Complications
- 90 day mortality
- Length of stay
- Definite pathological diagnosis- yes/no
- Overall diagnosis
- Change in treatment.

Quality Analyses

The overall quality of each study was judged independently by the two authors (TM and AM) including assessment of study type, internal validity, generalisability, heterogeneity and precision.

The quality and validity of each study was further assessed using the QualSyst tool for quantitative studies(45). This tool is based on the study design, method of population sampling, strategies of data collection and analysis, and how the conclusions were ascertained. The checklist and point scoring system are outlined in Table 2.1. The ensuring summary score is calculated as follows:

\[
\text{Total sum} = (\text{number of “yes”} \times 2) + (\text{number of “partials”} \times 1)
\]
\[
\text{Total possible sum} = 28 – (\text{number of “N/A”} \times 2)
\]
\[
\text{Summary score: total sum / total possible sum}
\]

Therefore, the best available score is 1 and the worst 0.
<table>
<thead>
<tr>
<th>Criteria</th>
<th>YES (2)</th>
<th>PARTIAL (1)</th>
<th>NO (0)</th>
<th>N/A</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
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<tr>
<td>Question / objective sufficiently described</td>
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<td>2</td>
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<td>Study design evident and appropriate?</td>
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<td>3</td>
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<tr>
<td>Method of subject/comparison group selection or source of information/input variables described and appropriate?</td>
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<tr>
<td>Subject (and comparison group, if applicable) characteristics sufficiently described?</td>
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<td>5</td>
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<td>If interventional and random allocation was possible, was it described?</td>
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<td>If interventional and blinding of investigators was possible, was it reported?</td>
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<td>If interventional and blinding of subjects was possible, was it reported?</td>
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<td>8</td>
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<tr>
<td>Outcome and (if applicable) exposure measure(s) well defined and robust to measurement / misclassification bias? Means of assessment reported?</td>
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<td>9</td>
<td></td>
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<td>Sample size appropriate?</td>
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<td>Analytic methods described/justified and appropriate?</td>
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<td>11</td>
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<td>Some estimate of variance is reported for the main results?</td>
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<td>Controlled for confounding?</td>
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<td>13</td>
<td></td>
<td></td>
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<tr>
<td>Results reported in sufficient details?</td>
<td></td>
<td></td>
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<tr>
<td>14</td>
<td></td>
<td></td>
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<tr>
<td>Conclusions supported by the results?</td>
<td></td>
<td></td>
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</tbody>
</table>

**Table 2.1: QualSyst study quality assessment checklist**
Synthesis of results

Where possible, estimates of effect were collated across the selected studies. Due to the wide heterogeneity and non-comparative nature of the studies, a simple proportion of each outcome of interest was calculated. No source data was available to perform more in-depth statistical analysis. Poor data quality meant no meta-analysis could be performed.
2.3 Results

Figure 2.1 presents a flow chart for full breakdown in the identification of suitable studies. Of the 50 papers for which full texts were obtained, 26 did not meet inclusion criteria due to being excluded on:

- Patient population size (<20) - 2 papers
- Patient population not ILD – 6 papers
- Surgical technique/ intervention – 4 papers
- No original data (either review or duplication of previous publication)- 4
- Language – 2 papers
- Outcome measures not reported/ poor data quality – 4 papers.
- Conference abstracts with insufficient data - 4 papers

24 studies from fourteen countries evaluating SLB in the diagnostic work up of ILD in 2600 patients were eligible for review (Table 2.2).

These included one randomised controlled trial (46) and two prospective series (47, 48) while the remaining publications were retrospective case series (3, 4, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68). 2163 of 2543 patients underwent VATS biopsy (One study did not report the breakdown between VATS and OLB). Only 8 series comprised of more than 100 patients.
Risk of bias assessment

As all but one of the studies available were case series, most of them retrospective and often small, the overall quality assessment of the assimilated data was assessed as moderate to poor, with a high risk of bias.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Country</th>
<th>Study design</th>
<th>Patient n=</th>
<th>VATS n=</th>
<th>Mean Age</th>
<th>QualSyst Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park et al (59)</td>
<td>2007</td>
<td>Korea</td>
<td>retrospective</td>
<td>200</td>
<td>150</td>
<td>58</td>
<td>1</td>
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<tr>
<td>Utz et al (67)</td>
<td>2001</td>
<td>USA</td>
<td>retrospective</td>
<td>60</td>
<td>16</td>
<td>63.4</td>
<td>1</td>
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<tr>
<td>Kreider et al (69)</td>
<td>2007</td>
<td>USA</td>
<td>retrospective</td>
<td>68</td>
<td>68</td>
<td>58</td>
<td>0.94</td>
</tr>
<tr>
<td>Morris et al (30)</td>
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<tr>
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<td>64</td>
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</tr>
<tr>
<td>Miller et al (46)</td>
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<td>Canada</td>
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<td>42</td>
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</tr>
<tr>
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<td>retrospective</td>
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</tr>
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<td>2013</td>
<td>Germany</td>
<td>retrospective</td>
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<td>61.4 (median)</td>
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<td>2009</td>
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<td>Country</td>
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<td>---------------</td>
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<td>---------------</td>
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</tr>
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<td>retrospective</td>
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<td>94</td>
<td>94</td>
<td>59</td>
<td>0.83</td>
</tr>
<tr>
<td>Blanco et al (51)</td>
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<td>retrospective</td>
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</tr>
<tr>
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<td>57.1</td>
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</tr>
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<td>Japan</td>
<td>retrospective</td>
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<td>retrospective</td>
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<td>retrospective</td>
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<td>n/r</td>
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<td>2009</td>
<td>Portugal</td>
<td>n/r</td>
<td>53</td>
<td>37</td>
<td>47.2</td>
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</tr>
</tbody>
</table>

**RCT** - randomised controlled trial; n/r - not recorded.

**Table 2.2:** Summary and characteristics of studies included ordered by QualSys score. Prospective trials and RCT shaded in grey.
Primary and secondary outcomes

Data synthesis on outcomes was not possible due to poor available data quality. Therefore, we provide a narrative synthesis.

Mortality

There was a wide variance in mortality. Data was available for 2598 patients (two were censored as lost to follow up) and mortality was variably recorded as in-hospital mortality, 30 day 30-day mortality or 90- day mortality. Overall, there were 128 deaths reported. Mean mortality was 4.9% with a wide range of 0- 22.4% (Table 2.3).
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Size n=</th>
<th>Mortality n=</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temes et al</td>
<td>67</td>
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<td>22.4</td>
</tr>
<tr>
<td>Utz et al</td>
<td>60</td>
<td>10</td>
<td>16.7</td>
</tr>
<tr>
<td>Knipscheer et al</td>
<td>69</td>
<td>8</td>
<td>11.6</td>
</tr>
<tr>
<td>Fibla et al</td>
<td>311</td>
<td>33</td>
<td>10.7</td>
</tr>
<tr>
<td>Kayatta et al</td>
<td>194</td>
<td>13</td>
<td>6.8</td>
</tr>
<tr>
<td>Lettieri et al</td>
<td>83</td>
<td>5</td>
<td>6.1</td>
</tr>
<tr>
<td>Blanco et al</td>
<td>171</td>
<td>10</td>
<td>5.9</td>
</tr>
<tr>
<td>Tiitto et al</td>
<td>76</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
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<td>103</td>
<td>5</td>
<td>4.9</td>
</tr>
<tr>
<td>Kreider et al</td>
<td>68</td>
<td>3</td>
<td>4.5</td>
</tr>
<tr>
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<td>73</td>
<td>3</td>
<td>4.2</td>
</tr>
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<td>200</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Luo et al</td>
<td>32</td>
<td>1</td>
<td>3.2</td>
</tr>
<tr>
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<td>151</td>
<td>4</td>
<td>2.7</td>
</tr>
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<td>Miller et al</td>
<td>42</td>
<td>1</td>
<td>2.4</td>
</tr>
<tr>
<td>Bando et al</td>
<td>94</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Guerra et al</td>
<td>53</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>Morris et al</td>
<td>66</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Samejima et al</td>
<td>285</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Bagheri et al</td>
<td>38</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fibla et al</td>
<td>224</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plones et al</td>
<td>44</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sonobe et al</td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Yamaguchi et al</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2.3 Reported mortality in SLB ordered by mortality
Complications

19 studies reported complications in 2038 patients. Some studies reported more than one complication per patient. Hence the total number of reported complications (333) exceeds the number of patients reported to have suffered a complication (318). Again, there was a wide variation regarding the definition of a complication and how it was reported. The most common complication was prolonged air leak, variably defined as prolonged chest drainage of more than five or more than seven days. (Table 2.4) Pneumothorax was the next frequently reported complication and could presumably be equated to prolonged air leak. Respiratory failure and need for post-operative ventilation were reported in 58 cases – 2.8%.
### Complications

<table>
<thead>
<tr>
<th>Complications</th>
<th>n=</th>
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<tbody>
<tr>
<td>Prolonged air leak</td>
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<tr>
<td>Pneumothorax</td>
<td>54</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>40</td>
</tr>
<tr>
<td>Respiratory failure/ need for LTOT</td>
<td>29</td>
</tr>
<tr>
<td>Post-operative need for ventilation</td>
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</tr>
<tr>
<td>Exacerbation of underlying lung disease</td>
<td>29</td>
</tr>
<tr>
<td>Wound infection</td>
<td>10</td>
</tr>
<tr>
<td>Haemothorax</td>
<td>8</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>8</td>
</tr>
<tr>
<td>Intra-operative complication</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td>79</td>
</tr>
</tbody>
</table>

Table 2.4: Complications. 19 studies reported complications (n=2038); 318 patients suffered complications

*LTOT – Long Term Oxygen Therapy*

Mean overall complication rate was 19.4% with a range from 7.1% to 65.7%. (Table 2.5). Notably there was a wide range of complications reported by some
papers and only what could be considered significant ones (death, prolonged air leak) ones by others illustrating the variability of data quality.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Size</th>
<th>Complications</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
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<td>21</td>
<td>65.7</td>
</tr>
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<td>Bando et al</td>
<td>94</td>
<td>48</td>
<td>51.1</td>
</tr>
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<td>Morris et al</td>
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<td>Yamaguchi et al</td>
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<td>6</td>
<td>20</td>
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<td>19.8</td>
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<td>13</td>
<td>19.2</td>
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<td>Miller et al</td>
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<td>17.9</td>
</tr>
<tr>
<td>Park et al</td>
<td>200</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
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<td>14</td>
<td>13.6</td>
</tr>
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<td>38</td>
<td>5</td>
<td>13.2</td>
</tr>
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<td>Rotolo et al</td>
<td>151</td>
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<td>11.7</td>
</tr>
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<td>311</td>
<td>36</td>
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<tr>
<td>Plones et al</td>
<td>45</td>
<td>5</td>
<td>11.2</td>
</tr>
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<td>Guerra et al</td>
<td>53</td>
<td>5</td>
<td>9.5</td>
</tr>
<tr>
<td>Lettieri et al</td>
<td>83</td>
<td>7</td>
<td>8.5</td>
</tr>
<tr>
<td>Samejima et al</td>
<td>285</td>
<td>20</td>
<td>7.1</td>
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</table>

Table 2.5: Overall complication rates ordered by descending frequency.

Length of Stay
Only nine studies reported mean hospital length of stay. A further three reported median length of stays (Rotolo et al 7 days, Blackhall et al 4, Sigurdsson et al 4) but these were not included in the calculation of the mean of 5.4 days. Range 2.5- 10.1 days. (Table 2.6)

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<td>151</td>
<td>7</td>
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Table 2.6 Mean Length of Stay post SLB in length of stay descending order.
Diagnostic Yield

Diagnostic yield was reported by 19 studies reporting a definite pathological diagnosis in 1845 out of 2150 patients (86%). Not all papers clearly defined definite pathological diagnosis and we discounted biopsies reported in vague terms such as interstitial fibrosis or inflammation when calculating the diagnostic yield. The exact break down of diagnosis was not always available and therefore the quoted diagnostic hit rate could not always be verified. Mean diagnostic yield as a percentage was 89% and a range of 70-100%.

(Table 2.7)
<table>
<thead>
<tr>
<th>Author</th>
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<th>Definite Pathological Diagnosis n=</th>
<th>Diagnostic Yield %</th>
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</thead>
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<td>83</td>
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<td>32</td>
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<td>Miller et al</td>
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<td>42</td>
<td>100</td>
</tr>
<tr>
<td>Temes et al</td>
<td>68</td>
<td>68</td>
<td>100</td>
</tr>
<tr>
<td>Yamaguchi et al</td>
<td>30</td>
<td>30</td>
<td>100</td>
</tr>
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<td>Bando et al</td>
<td>94</td>
<td>91</td>
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<td>64</td>
<td>62</td>
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<td>151</td>
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Table 2.7: Quoted diagnostic yield, ordered highest to lowest.
Treatment Change
Eight studies recorded treatment change following SLB in a total of 588 patients out of 869. Mean percentage change was 60%. Interestingly, even a non-diagnostic biopsy did lead to treatment changes in some cases (Table 2.8).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study size n=</th>
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<th>% of Treatment Change</th>
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</thead>
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<td>Tiitto et al</td>
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<td>n/r</td>
<td>58</td>
<td>77</td>
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<td>Temes et al</td>
<td>68</td>
<td>68</td>
<td>42</td>
<td>62</td>
</tr>
<tr>
<td>Yamaguchi et al</td>
<td>30</td>
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<td>17</td>
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<td>46</td>
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</table>

Table 2.8: Treatment change following SLB, ordered highest to lowest % of change

Other Outcome Measures
Another outcome measure outlined in the systematic review protocol included overall diagnostic yield (taking into consideration the clinical, radiological and pathological presentation) but not enough data was recorded in any of these studies to report on this outcome measure.
2.4 Discussion

This systematic review set out to specifically examine the morbidity and mortality associated with SLB in the diagnosis of ILD in the modern VATS era. Overall, the data obtained was heterogeneous with wide variation in reported outcome measures and therefore a comprehensive analysis of the data was not possible. However, it is still striking that SLB in ILD carries a significant morbidity and mortality for a diagnostic test.

Not only have surgical approaches changed in the last 20 years but also our understanding and sub-classification of ILDs. Fibla et al have developed a risk stratification mortality score that takes into consideration pre-biopsy patient criteria such as: age >67 years; preoperative intensive care unit admission; immunosuppressive treatment; open surgery (76). However, it is clear from published data evaluating outcomes after lung cancer surgery and general abdominal surgery that interstitial fibrosis and IPF predispose to acute exacerbation and increased mortality (79, 80, 81). Hence while careful risk stratification prior to SLB would undoubtedly avoid some of the associated mortality it would be unable to eliminate the inherit risk of an, yet undiscovered, IPF diagnosis. Neither does it change the diagnostic conundrum of patients too unwell to undergo surgery.

SLB is associated with a considerable health care burden with an average length of stay of 5.4 days which is at least in part due to the two most common complications- prolonged air leak and pneumothorax. The diagnostic yield was overall good with a mean diagnostic yield of 89%, ranging from 70-100%. However, there remains some doubt as to whether large case series reporting
100% definite pathological diagnosis have included ambiguous reports such as “interstitial fibrosis” in their reports. Change in treatment course was the most surprising outcome with a mean percentage change was 60% (CI 90% 0.35-0.87) with changes even taking place when no definite pathological diagnosis could be reached. There are two possible explanations for this: either the biopsy excluded some candidates of the differential diagnosis shortlist which allowed a consensus diagnosis to be made or clinicians decided on an empirical change of treatment despite no clear pathological diagnosis having been made.

Limitations

Overall, the data quality of the papers included in this systematic review is poor, with a high risk of reporting bias and, therefore, interpretation of the results in this study should be guarded.
METHODS

The PUBMED search was repeated with a date range amendment to 01/07/2015 – 31/12/2020.

PUBMED search accessed 30/10/2020.

((((((("histopathology") OR "video assisted thoracic surgery") OR "video assisted thoracoscopic surgery") OR "VATS") OR "open lung biopsy") OR "surgical biopsy") OR "surgical lung biopsy") AND (((("diffuse parenchymal lung disease*") OR ILD) OR "interstitial lung disease*") OR "parenchymal infiltrate*") OR "pulmonary fibrosis") OR "lung fibrosis") AND (("2000/07/01"[PDat] : "2020/12/31"[PDat])))) AND english[Language] AND (hasabstract[text] AND English[lang])

The same exclusion and inclusion criteria as in the original systematic review were applied by a single reviewer. No formal quality scoring was performed.

RESULTS

PUBMED search returned 514 results that were abstract screened for inclusion and exclusion criteria. 22 publications were identified for full text screen. Of those 11 were excluded on the following reasons:

- Full text not available: 4
- Not reporting relevant outcome measures: 3
- Duplicate data: 1
- Paper included in original analysis: 1
- Patient population (ITU): 1
- Surgical Procedure technique (transdiaphragmatic approach): 1

The remaining eleven studies reported SLB outcomes from eight countries and were all retrospective. Eight were case series (six single centre) reporting outcomes for a total of 731 patients. The remaining three studies were large healthcare database-based cohort studies reporting on a total of 37899 patients. (See Table 2.9 for details)
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Study Design</th>
<th>Patients n=</th>
<th>Single or multicentre</th>
<th>Mean Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biancosino et al</td>
<td>2016</td>
<td>Germany</td>
<td>case series</td>
<td>97</td>
<td>single</td>
<td>51</td>
</tr>
<tr>
<td>Cherchi et al</td>
<td>2020</td>
<td>Italy</td>
<td>case series</td>
<td>99</td>
<td>single</td>
<td>66</td>
</tr>
<tr>
<td>Fisher et al</td>
<td>2019</td>
<td>Canada</td>
<td>cohort</td>
<td>3057</td>
<td>multicentre</td>
<td>n/r</td>
</tr>
<tr>
<td>Hutchinson et al</td>
<td>2016</td>
<td>USA</td>
<td>cohort</td>
<td>32022</td>
<td>multicentre</td>
<td>n/r</td>
</tr>
<tr>
<td>Hutchinson et al</td>
<td>2016</td>
<td>England</td>
<td>cohort</td>
<td>2820</td>
<td>multicentre</td>
<td>n/r</td>
</tr>
<tr>
<td>Jeon et al</td>
<td>2018</td>
<td>Korea</td>
<td>case series</td>
<td>25</td>
<td>single</td>
<td>61.2</td>
</tr>
<tr>
<td>Pompeo et al</td>
<td>2019</td>
<td>multinational</td>
<td>case series</td>
<td>112</td>
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<td>60</td>
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<tr>
<td>Ravaglia et al</td>
<td>2016</td>
<td>Italy</td>
<td>case series</td>
<td>150</td>
<td>single</td>
<td>59</td>
</tr>
<tr>
<td>Sugino et al</td>
<td>2019</td>
<td>Japan</td>
<td>case series</td>
<td>143</td>
<td>single</td>
<td>64 (median)</td>
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<tr>
<td>Tibana et al</td>
<td>2020</td>
<td>Brazil</td>
<td>case series</td>
<td>50</td>
<td>single</td>
<td>64</td>
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<tr>
<td>Vaszar et al</td>
<td>2018</td>
<td>USA</td>
<td>case series</td>
<td>55</td>
<td>multicentre</td>
<td>77 (median)</td>
</tr>
</tbody>
</table>

Table 2.9: Study characteristics (2015-2020)

n/r – not recorded
Outcome measures are grouped for case series and cohort studies as they differ in the outcomes reported. None of the studies reported change in treatment following SLB; seven reported complication rates; seven reported histopathological diagnosis and four length of stay.

The primary outcome, mortality rate, was variably reported as in hospital mortality, 30-day mortality, 90-day mortality, or not further defined. Studies that did not report on mortality were excluded from the analysis. Table 2.10 summarises outcome data for the retrospective case series.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study size n=</th>
<th>Surgical approach</th>
<th>Mortality* %</th>
<th>Complications %</th>
<th>Diagnostic yield %</th>
<th>Length of stay (days - mean)</th>
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</thead>
<tbody>
<tr>
<td>Ravaglia et al</td>
<td>150</td>
<td>VATS</td>
<td>2.7</td>
<td>12.7</td>
<td>98.7</td>
<td>6.1 (median)</td>
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<tr>
<td>Sugino et al</td>
<td>143</td>
<td>VATS</td>
<td>0</td>
<td>18</td>
<td>Not reported§</td>
<td>n/r</td>
</tr>
<tr>
<td>Pompeo et al</td>
<td>112</td>
<td>non-intubated VATS</td>
<td>0</td>
<td>7.1</td>
<td>96</td>
<td>2.5</td>
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<tr>
<td>Cherchi et al</td>
<td>99</td>
<td>non-intubated VATS</td>
<td>1</td>
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<td>98</td>
<td>1.3</td>
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<td>VATS</td>
<td>1</td>
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<td>100</td>
<td>n/r</td>
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<tr>
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<td>55</td>
<td>not reported</td>
<td>9.7</td>
<td>not reported</td>
<td>96.4</td>
<td>n/r</td>
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<tr>
<td>Tibana et al</td>
<td>50</td>
<td>not reported</td>
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<td>96</td>
<td>n/r</td>
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<tr>
<td>Jeon et al</td>
<td>25</td>
<td>VATS</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2.10: Case series outcome data (2015-2020)

§ overall diagnostic yield after MDT reported as 100%, n/r – not reported

* Mortality reported as in-hospital or 30-day. Sugino and Vaszar additionally report 90-day mortality as 0.7 and 15.4% respectively.
None of the cohort studies reported mean age, length of stay or diagnostic yield. The SLB approach used was not consistently described. Only one study stated complication rates.

The smallest of the cohort studies investigated the mortality and complications associated with SLB in England between 1997-2008, identifying 2820 patients. Overall complication rate was reported as 13.9%. Observed in-hospital, 30-day and 90-day mortality was 1.7%, 2.4% and 3.9% overall; with a lower mortality for elective cases (1.0%, 1.5%, and 2.8%) than for non-elective cases (4.6%, 6.3%, 8.8%) (86).

A similar difference in emergency versus elective procedures is also reported by the second largest study investigating the outcomes for adult patients with ILD undergoing SLB between 2001 and 2014 through interrogation of population-based data available at the Institute for Clinical Evaluative Sciences in Ontario, Canada. Of 3057 procedures performed the 30-day overall mortality was 7.1% with marked differences between non-elective and elective patients (20.2% and 1.9%, respectively) (84).

The largest cohort study looked at 32022 cases (albeit this is based on weighted data and hence mortality outcomes are estimates, not observed) performed between 2000-2011 in the United States of America. The results are again very similar with an overall in-hospital mortality of 6.4% (95% CI, 6.1–6.7%), elective: 1.7% (95% CI, 1.5–1.9%), non-elective 16.0% (95% CI, 15.2–18.8%) (85).
2.6 DISCUSSION

The original systematic review in 2015 and the follow-up update in 2020 illustrate the pitfalls of single centre, retrospective case series – poor data quality, heterogeneity of reporting and high risk of bias. Several studies report their good safety profile with low mortality rates while those are often based on case series with less than 100 patients.

Our original review found a mean overall mortality of 4.9% which is broadly in keeping with the findings of the large database driven cohort studies that added much needed unbiased evidence to the available data.

The evidence is now clear that non-elective SLB is extremely high risk though does have its role even in a critical care setting for guiding treatment decisions, including withdrawal of care (93).

The awareness that elective SLB, in previously relatively well ILD patients, carries significant risk in the context of a diagnostic procedure has been growing over the last few years. This risk increases with an underlying diagnosis of IPF and advancing age (92). Emerging data on the use of anti-fibrotics in non-IPF patients (94) will further decrease the need for SLB in ambiguous diagnosis.

However, as the second re-iteration of this literature search has shown, there are continuing advances in SLB techniques (and alternative biopsy methods) that might improve complication and mortality rates. During standard VATS procedure the patient is anaesthetised using a double lumen endo-tracheal tube to allow single lung ventilation, which is thought to be the main driver for post-surgical acute exacerbation of ILD (which incidentally can occur in fibrotic ILD patients after any surgical procedure requiring ventilation, not only thoracic
surgery (81)). Awake VATS under deep sedation has been pioneered in Spain and Italy and the published complication rates, coupled with reduced length of stay and post-operative thoracic pain are encouraging (83, 88, 95). Disappointingly, not much emphasis has been put on whether SLB changes therapeutic treatment and, crucially, outcome. While some attempts have been made (notably by Blackhall et al (70)) it very much remains an unanswered question with likely shifting goalposts in view of shifting treatment indications.

**LIMITATIONS**
This systematic review was mainly limited by poor data quality, the retrospective nature of nearly all studies and the heterogeneity of trials and their populations. However, the large population-based cohort studies provide at least consistency with regards to mortality data.

**CONCLUSION**
SLB carries a significant mortality and morbidity risk, even in elective patients. Careful weighing up of risks and benefits for each individual patient as well as involvement of the specialist ILD MDT (including thoracic surgery) remains imperative.
Chapter 3

Exploring the current UK practice of the MDT in the diagnostic work up of ILDs –

A Benchmarking Survey
3.1 Introduction

It is difficult to overstate the importance of the Multidisciplinary Team (MDT) (and MDT meeting) in the diagnosis and management of ILD. It forms an integral part of all specialist ILD services. As has been outlined in chapter 1, the diagnosis of ILD can be challenging at times. No single diagnostic test can be considered to provide a definite, confident diagnosis in almost all ILDs. Therefore, a consensus diagnosis reached by an MDT with expertise in ILD is now considered the gold standard. The MDT can integrate all available data at several stages of the diagnostic work up. This does not only improve inter-observer agreement and diagnostic confidence(34) but may also prevent unnecessary invasive procedures such as surgical biopsies while conversely identifying patients in whom a biopsy may effectively contribute to the diagnosis. Current NICE guidelines recommend that IPF should only be diagnosed by MDT consensus(11). They also recommend a minimum MDT composition of one consultant respiratory physician, one consultant radiologist, an ILD specialist nurse and an MDT co-ordinator all of whom should have expertise in ILD. When invasive diagnostics are considered a consultant histopathologist and, if appropriate, a consultant thoracic surgeon should also form part of the MDT. There is some evidence that involving more than one clinician of the same specialty in a MDT increases inter-observer agreement between specialties(35).

The ILD, and especially the idiopathic interstitial pneumonias (IIP) of which IPF is the most common, landscape has changed quite dramatically over the last decade. Firstly, due to discreditation of triple therapy with prednisolone, azathioprine and N-acetylcysteine as the results of the Panther trial (10) and
then with the arrival of the anti-fibrotics pirfenidone and nindetanib. These developments further shifted the onus of diagnosis and therapy away from district general hospitals (DGHs) to specialist centres, especially in England with its funding restrictions on these costly drugs.

In 2013 ILD Services became part of the recently established nationally commissioned specialist services by NHS England. Service specifications are reviewed by the respiratory Clinical Reference Group to ensure they take into consideration all the relevant guidelines and evidence, in this case the 2013 NICE IPF guidelines(11). It is against this set of service specifications – which outline optimal service provision – that all specialist ILD centres in England are assessed. To quote from a pertinent section in the NHS England ILD Service Specification 2018(96):

“The overall aim of the specialist service is to ensure equality of patient access to multi-disciplinary team diagnosis, to guarantee that patients with ILD have equal access to current treatment modalities and that their disease-specific management plans are drawn up following MDT assessment at regional specialist units.”

Part of the specified service objectives are “to provide a specialist multi-disciplinary service for diagnosis” and “and to “enable integration of clinical services with clinical trials and translational research to ensure on going developments in the care of individuals with these rare diseases.”

Outcome measures for service performance include % patients discussed at ILD MDT and access to clinical trials.
At the time of the implementation of these service changes the 2011 ATS/ERS (13) consensus statement estimated that about two thirds of cases of IPF could be diagnosed based on typical radiological findings of UIP and clinical picture. Surgical lung biopsies (SLB) were, and are, the current gold standard for obtaining histological material in the diagnosis of IIPs. Therefore, around a third of patients with suspected IPF would require SLB to confirm or refute the diagnosis. We estimate from two surveys that only 7.5%-12% of suspected IPF patients undergo SLB in the UK (28, 29). This may reflect the reluctance of clinicians to refer patients for a procedure associated with significant mortality and morbidity. The annual IPF incidence per 100,000 population in the UK is 7.44(97) which translates to an estimated 5000 new IPF diagnosis per year. If ATS/ERS diagnostic guidelines were to be followed one would expect at least 1500 SLB per year. This does not consider SLB performed in other suspected IIPs.

The Society for Cardiothoracic Surgery in Great Britain and Northern Irelands performs a yearly national audit across all cardiothoracic centres in both countries which includes returns on wedge resection performed for diagnosis. In 2013/14 928 VATS wedge resections were performed for diagnosis. However no differentiation is made between diffuse disease and multiple nodules and no data is available on the eventual diagnosis achieved(98). Thoracic surgical outcome data focuses on lung cancer and other malignancy resection rates and there is no further data available for benign lung biopsies beyond the 2013/14 report.
Since their inception in 2013 specialist ILD services have had seen an ever-expanding patient case load. The incidence of IPF in the UK is rising (97) and demographic changes are likely to accelerate this trend.

3.2 AIMS

This survey had the following aims:

- To benchmark current MDT provision
- To benchmark current ILD center activity with regards to SLB and clinical trials
- To gather supporting evidence for services to lobby for more resources

3.3 METHODS

A questionnaire was developed to map current practice in specialist ILD centres in the UK and surveyed all NHS England commissioned Specialist ILD Centres (23) as well as known centres of expertise in Scotland (3), Wales (1) and Northern Ireland (1) on their current diagnostic MDT practice. A total of 20 questions assessed the workforce composition and frequency of meetings. Their workload was also evaluated, and we asked them to identify areas that required improvement.

The survey was emailed to the 28 clinical leads. Responses were collected anonymously over a 6-month period (August 2015 to February 2016) via an online platform. Permission for publication was sought at the same time.

See item list below:
ILD MDT provision in the UK- benchmarking survey of all specialist ILD centres in the UK

Questionnaire sent to all lead consultants of ILD centres.

1) Are you the lead consultant for an ILD service in
   a) Approved National ILD referral centre in England.
   b) Scotland
   c) Wales
   d) NI

2) Who regularly attends your MDT?
   - Respiratory consultants
   - Respiratory SpRs
   - Consultant radiologist – with/without a specialist interest in ILD
   - Consultant pathologist – with/without a specialist interest in ILD
   - Consultant rheumatologist
   - ILD specialist nurse
   - Thoracic surgeon
   - MDT coordinator
   - Other (please specify)

3) Who co-ordinates your MDT?
   - Dedicated MDT co-ordinator
   - ILD lead consultant.
- Other respiratory consultant
- Respiratory SpR
- ILD specialist nurse
- Medical secretary
- Other (please specify)

4) Do other hospitals participate in your MDT yes/no
   If yes, select all that apply.
   - attend in person.
   - via video link
   - refer patients for discussion in writing.

5) What percentage of patients discussed at your MDT attend your centre in person for a review?
   - <25%
   - 25-50%
   - 50-75%
   - > 75%

6) Frequency of MDT
   - Twice weekly
   - Weekly
   - Fortnightly
   - Monthly
   - Ad hoc
7) On average, how many patients are discussed per MDT.
- <10
- 10-20
- 20-30
- >30

8) How much time is allocated per MDT?
- <1h
- 1-2h
- 2-3h
- >3h

9) Are all new ILD referrals discussed?
- Yes, all.
- Almost all (>75%)
- The majority (>50%)
- a proportion (25-50%)
- only the most challenging cases (<25%)

10) Are all patients considered for anti-fibrotic therapy (i.e., pirfenidone) discussed in MDT prior to initiating therapy?
- Yes, all.
- Almost all (>75%)
- The majority (>50%)
- a proportion (25-50%)
- only the most challenging cases (<25%)

11) Are all patients considered for immunosuppressive therapy other than oral steroids (i.e., cyclophosphamide, MMF etc) discussed in MDT prior to initiating therapy?
- Yes, all.
- Almost all (>75%)
- The majority (>50%)
- a proportion (25-50%)
- only the most challenging cases (<25%)

12) In your opinion is the available MDT time sufficient?
- Yes, exactly the right amount of time.
- No, we manage but are pushed to get through all cases.
- No, with more time we would be able to discuss all new cases and not just the challenging ones.
- No, we struggle to discuss even the challenging cases.

13) If you answered No to question 11 what the main reasons for insufficient MDT time are (select all that apply)
- No dedicated funding for MDT
- Availability of respiratory consultants
- Availability of consultant respiratory radiologists
- Availability of other specialties participating in MDT
- Availability of facilities (conference room, teleconference facilities)
- Availability of admin support (MDT co-ordinator, typing of MDT outcomes etc)
- Other (please specify)

14) In the last year, how many patients have been referred for surgical lung biopsy by your centre?
- None
- <5
- 5-10
- 11-15
- 16-20
- >20

15) In the last 3 months how many parenchymal histopathology specimens have you reviewed in your MDT? (i.e., surgical lung biopsies and transbronchial biopsies; not EBUS or BAL)?
- None
- 1-5
- 6-10
- 11-15
- 16-20
16) Do you enrol any patients discussed at your MDT into clinical trials? (Select all that apply)
- No
- Yes, we enrol patients into trials co-ordinated by other centres.
- Yes, we are the co-ordinating centre for a single centre clinical trial.
- Yes, we are the co-ordinating centre for a multi-centre clinical trial.

17) Is there a local tariff in place to fund MDT discussion of ILD patients?
- Yes/No

18) Would a local or national tariff for ILD MDT discussion improve MDT provision in your centre?
- No- no improvement needed.
- No- the restraints are not financial.
- Yes- it would allow us to employ necessary support staff (MDT co-ordinator, admin)
- Yes- it would increase available consultant time.
- Other (please specify)

19) What steps have you taken so far to improve your ILD MDT?
- Free text
20) Please enter any additional comments regarding ILD MDT provision you may have.

- Free text
3.4 RESULTS

26 specialist centres replied: 3 centres in Scotland, 1 in Wales, 1 in Northern Ireland and 23 in England.

Consultant radiologists with a specialist interest in ILD and respiratory consultants attended the MDT regularly at every specialist service surveyed. A specialist ILD nurse was regularly present at 83% of meetings while an MDT co-ordinator was only present at 26%. Please see Figure 5.1 for detailed break-down of attendance at MDT.

Figure 3.1: Specialist ILD MDT composition

The majority of ILDs are co-ordinated by the ILD lead consultant (57%); only 17% are organised by a dedicated MDT co-ordinator or the ILD specialist nurse, while 26% are arranged by a medical secretary (26%). Other centres share the burden between consultants and registrars (with everyone bringing individual cases to discuss – 2 centres), involve the research team (research nurses and clinical fellows – 1 centre).
22% of centres report no participation in their specialist MDT from other hospitals, while others host external clinicians either in person (44%) or via video-link (26%). 65% of ILD MDTs also discuss written external referrals. 83% of centres report that most patients discussed (>75%) also attend the specialist service in person for review; 17% report that 50-75% of MDT patients are seen in person. MDT are held weekly (44%), fortnightly (17%), or monthly (39%). Most MDTs discuss between 10-20 patients per session and last between 1-2 hours (see Figures 3.2 and 3.3)

![Figure 3.2: Average number of patients discussed per MDT.](image)
Most centres review all or almost all referred ILD patients; however, 3 centres reported to only discuss the most challenging cases. (Figure 3.4)

Figure 3.3: Average length of MDT meeting

Figure 3.4: Proportion of referred cases discussed.
87% report that all patients considered for antifibrotic therapy are discussed (as per national guidelines). However, one centre reports only to be discussing the most challenging cases.

The proportion of patients discussed for immunosuppressant therapy is lower than for anti-fibrotic therapy (Figure 3.5).

**Figure 3.5: Proportion of patients being considered for immunosuppressive therapy discussed at MDT.**

None of the responding centres thought the time available for discussion at MDT was sufficient with 35% stating that this is the reason why they cannot discuss all cases. Lack of dedicated MDT funding and lack of available specialist radiology consultant time were mentioned as the main reason. (Figure 3.6)
The next part of the survey focused on the SLB referral rate from the specialist MDT and how often relevant histology was reviewed. (Table 3.1 and Table 3.2).

All but 2 centres were enrolling patients into clinical trials, either co-ordinated by other centres (58% of centres), or as a single (35%) or multi-centre co-ordinating site (39%).
In the last year, how many patients have been referred for surgical lung biopsy by your centre?

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>&lt;5</td>
<td>16.0%</td>
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<td>5-10</td>
<td>32.0%</td>
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<tr>
<td>11-15</td>
<td>24.0%</td>
<td>6</td>
</tr>
<tr>
<td>16-20</td>
<td>16.0%</td>
<td>4</td>
</tr>
<tr>
<td>&gt;20</td>
<td>8.0%</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3.1: Frequency of referral for surgical lung biopsy
In the last 3 months how many parenchymal histopathology specimens have you reviewed in your MDT? (i.e., surgical lung biopsies and transbronchial biopsies; not EBUS or BAL)

<table>
<thead>
<tr>
<th>Answer Options</th>
<th>Response Percent</th>
<th>Response Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4.0%</td>
<td>1</td>
</tr>
<tr>
<td>1-5</td>
<td>36.0%</td>
<td>9</td>
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<td>6-10</td>
<td>28.0%</td>
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<td>11-15</td>
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<td>16-20</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>&gt;20</td>
<td>20.0%</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3.2: Frequency of review of histopathological specimens
All but one centre reported that there was no local tariff for MDT funding in place (no national tariff existed at the time), and they all agreed that funding would improve MDT provision by allowing employment of necessary support staff and increasing consultant time availability.
The final two questions of the survey were free text. We have included a selection of quotes in the results to portray the difficulties and mood in the specialist ILD community around the time of the Specialist Commissioned Service implementation:

Question 19:

What steps have you taken so far to improve your ILD MDT?

“We have just carried on.”

“trying to get funding for MDT coordinator, tried to get tariff for MDT”

“I meet with the specialist nurse 2 weeks in advance to ensure all patients on the MDT list have had all appropriate investigations and investigations done elsewhere are tracked and made available for the meeting. This ensures the best use of MDT time “

“recent business case to get funding for more radiology time, pathology time, more nursing time and MDT coordinator and local implementation being looked at for ILD MDT fee to help fund all this. It’s been a tough battle but finally moving forward now”

“We have struggled to get the MDT funded by management and have had to rely on the goodwill of our radiology colleagues”

“we are trying to develop teleMDM but funding is a problem”
“Discussions with management, limited benefit. Discussions with local commissioners, no benefit. Discussion at regional respiratory meeting – plan for network agreed. Discussion with commissioners commenced but stopped July 2015 as manager supporting had contract terminated”

“Publicity about its existence. Referral and output documentation. Appointment of ILD Nurse specialist. Agreement for radiology sessional time”

“Just appointed ILD Specialist Nurse (3 days a week). In Scotland she is only the 2nd ILD nurse, so huge gaps in terms of provision across our country”

“All outcomes are recorded digitally and filed in patient notes. There is a Trust support in principle for a business plan to employ an ILD MDT coordinator and an ILD nurse. This is in hand”

“Use research staff to co-ordinate; use standard program for referring hospitals”
Question 20:

Please enter any additional comments regarding ILD MDT provision you may have.

“I would like more than one MDT per month, the demands on the service have grown enormously over the last 2 years, particularly since the advent if antifibrotic medication, with no associated increase in time and support of the MDT. Is not uncommon for patients not to be discussed in the MDT due to lack of time, so have to be discussed elsewhere, such as the X-ray meeting, this is suboptimal. 2 of the 3 respiratory consultants can only commit to 3/12 MDTs per year. This is due to other commitments in their job plans, the low value of the MDT in terms of Pas and low financial value of the MDT to the Trust. We do not receive an MDT tariff per patient discussed in MDT, it is factored into a new patient tariff. Therefore the trust does not receive any remuneration if we do not see the patient in our clinic”

“We run a weekly mini-MDM at which every patient in the local speciality clinic and selected patients from local DGHs are presented. We run a 3-monthly ‘maxi-MDM’ at which predominantly biopsied patients locally and tertiary referrals are discussed. We can’t justifies have a pathologist at a weekly MDM”
“grossly inadequate throughout UK in general depend on whether individual trusts can be convinced that supporting an ILD service is good for them or not! Difficult times but slowly improving at least at my site”

“Audit of MDT showed change in diagnosis in ~40% of referrals. This was used to build case for additional 1 hr MDT which we plan to start later this year. Obtaining funding for this from local trust has been challenging and has only been possible as we charge a locally agreed tariff for MDT. We have been unable to secure consultant Rheumatology time for MDT”

“Funding constraints – have 0.5 WTE MDT coordinator, funded by industry – will need to persuade trust to fund. Not currently adequate to support full BTS databasing and ILD minutes.”

“Not really viewed as a priority by anyone not immersed in ILD, no funding for co-ordinator, no standard means of recording outcomes”

“we need a national tariff!”

“A tortuous path but progress is slowly being made.”
“Agree lung cancer model is what we should be aiming for, discussing all patients with appropriate administrative support and time in job plans for those involved”

“We have had major difficulty with our MDT because of the lack of tariff for this which has resulted in lack of support from non-respiratory specialists to expand our service. Our pathologists join monthly”
3.5 Discussion

The advent of novel anti-fibrotic therapies and, in the case of England, the establishment of specialist, nationally commissioned Interstitial Lung Disease (ILD) centers, has led to an increased workload for specialist Multidisciplinary Team (MDT) meetings. It is clear from the survey response that these centers are under considerable strain to deliver the required specialist service.

All respondents agreed that the available MDT time was insufficient. The most common reasons were cited as: lack of dedicated MDT funding (83%), lack of sufficient respiratory radiologist consultant time (78%) and lack of dedicated administrative support (61%).

In 96% of cases there is no local tariff in place to fund MDT discussion and all respondents agreed that a dedicated tariff would improve MDT provision.

Two thirds of specialist centres refer less than 15 patients per year for surgical lung biopsy (see Table 3.1) and therefore one could hypothesize that the bulk of diagnostic SLB referrals originate from non-specialised centres. A similar observation has been made by the recently established German IPF registry INSIGHTS-IPF (99).

This evidence is further substantiated by the fact that many more histology samples get reviewed by specialist MDTs than patients referred by specialist centres for surgery. Furthermore, the number of biopsies does not align with the audit by The Society for Cardiothoracic Surgery in Great Britain and Northern Irelands which reported 928 diagnostic VATS wedge resections performed in 2013/14 (98). As the cardiothoracic audit data does not report final diagnosis it might be overestimating the VATS rate for benign diagnostics.
as at least a proportion of the histology will be malignant or be for non ILD disease and hence will never be discussed in the setting of an ILD MDT. It is imperative that patients are discussed in a specialist ILD MDT prior to surgical referral for consideration of VATS biopsy to avoid unnecessary surgery and ensure adequate follow-up and audit of these invasive and potentially harmful, diagnostic procedures.

3.5.1 LIMITATIONS

This benchmarking survey did not reach all specialist centres and can only provide a snapshot in time and is therefore already out of date. The challenges of poor funding in the UK’s ILD services have however not gone away. With the licensing of nintedanib for progressive fibrosing ILD and the removal of the upper FVC limit by NICE in 2023 the patient case load for antifibrotic therapy and hence pressure on specialised ILD services and MDTs has further increased. The surgical audit data is of only limited utility as it does not differentiate between “diffuse diseases” and “multiple nodules” and does not record final pathological diagnosis. More robust audit data mapped to referring centres and pre and post biopsy diagnosis would be needed to make clear conclusions regarding the utilisation of SLB in ILD.

3.6 Conclusion

Specialist ILD MDTs can concentrate a high level of expertise and allow patients access to high quality diagnostic and therapeutic intervention as well
as to vital clinical trials. They are, however, under considerable strain due to lack of funding and administrative support. A dedicated funding stream for this specialist service would be beneficial.

At the time of this survey, SLB appeared to be underutilised in the diagnosis of ILDs in the UK. There appears to be a disparity between specialist and non-specialist ILD centres however the data is not sufficient to draw any clear conclusions.

It would be of interest to repeat the above survey focusing on MDT workload as opposed to histopathological sampling which has been further relegated in the diagnostic hierarchy in recent years. Therefore the “under-utilisation” found at the time of this survey is likely not an ongoing concern due to a change in clinical diagnostic guidelines and improved radiological diagnostics.
Chapter 4  Transbronchial Cryo Lung Biopsy – A literature review
4.1 Introduction

The first transbronchial cryobiopsy (TBCB) paper was published by Dr Hetzel’s group in Tubingen, Germany in 2009 (27). Since then, several groups- mainly concentrated in continental Europe and the USA- have published large case series.

There is mounting evidence and awareness of the risks involved with the procedure as well as the potential diagnostic gain. Nonetheless it remains somewhat controversial and has not been recommended in recently updated diagnostic guidelines (100).

This chapter sets out the current knowledge base with regards to diagnostic yield and complication profile.
4.2 METHODS

I performed a literature review by searching PubMed and ATS (American Thoracic Society) and ERS (European Respiratory Society) conference abstracts for the search term.

((“transbronchial cryobiopsy”) AND ((“ILD”) OR (“Interstitial Lung disease” (OR “diffuse parenchymal lung disease”)))

from 2009 to 2020. Reviews, opinion pieces, individual case reports, conference abstracts and series including other patient populations (for example transplant patients or Acute Respiratory Distress Syndrome ARDS) without reporting separate outcome measures for ILD only were excluded.

Data was extracted to record histological diagnostic yield, overall complication rate, rate of moderate or severe bleeding, rate of pneumothorax, rate of pneumothorax requiring insertion of intercostal chest drain (ICD), and 30-day mortality.

Analysis of exact patient numbers and complication rates is somewhat hampered as several groups have sequentially republished expanding case series, leading to a repetition of data without this being always apparent (see for example Ravaglia et al 2016, 2017 and 2019(89, 101, 102)). I have therefore censored data published by the same group if the patient recruitment time span overlapped with a later published case series and have only included the most recent data.
All statistical analysis had been performed on PRISM9 (Graphpad, San Diego, 2020)
4.3 RESULTS

A total of 39 relevant papers (n=35) and abstracts (n=4) were identified reporting on the outcomes of 3981 patients. (Table 4.1). Just over half of the trials were conducted prospectively (20, 51.3%), including two recent studies comparing the histological and MDT diagnostic yield of TBCB directly with SLB (103, 104) adding to the existing retrospective data trying to answer this important question (101, 105, 106).

In recent years reported patient numbers have increased steadily with the largest case series published in 2019 comprising 699 patients. (102)
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<th>Journal or Conference Publication</th>
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Table 4.1: TBCB published papers and conference abstracts
Diagnostic Yield

Histopathological diagnostic yield was reported by 34 of the included studies representing 2911 patients. The overall reported mean was 71.9% (SD14.4, 95% CI 66.9- 76.9) while the median was 73.7% (IQR 63.6-85.2%). Pooling all available data to calculate an overall weighted mean result in 75.4%, meaning 2194 out of 2911 patients who underwent TBCB received a histopathological diagnosis. (See Table 4.2)

Complications

Main complication rates are summarised in Table 4.2

There were 15 reported deaths within 30-days following on from the procedure, giving an overall reported 30-day mortality of 0.38%. This overall mortality is the same as reported by the largest available case series comprising 699 patients (102). In three publications, authors specifically commented that their reported deaths were unrelated to the procedure itself (121, 137, 142).

Complications were not reported by one of the papers included as only the abstract was available for review (102). Most focused their complication reporting on the two main known adverse events linked to TBCB: endobronchial bleeding and pneumothorax. Some study designs, such as sequential TBCB and SLB (103), prohibited the reporting of pneumothorax rates.
Pneumothoraces occurred in 371 out of 3700 procedures (10.0%). 179 of those (48.2%) required insertion of an Intercostal Chest Drain (ICD). Nine studies did not specify whether ICD insertion was required so this is likely an underestimate. One paper did not report pneumothorax rate. In two studies this outcome measure was not applicable due to sequential SLB at same sitting to TBCB with post-operative pneumothorax and ICD insertion the norm. The overall reported mean for pneumothorax occurrence in all studies was 9% (SD 7.8, 95% CI 6.4-11.7) while the median was 7.1% (IQR 2.5-14.9%).

Moderate to severe bleeding occurred in 384 out of 3325 cases (11.5%). The sub-classification or grading of bleeding into minor, moderate or major bleeding is undertaken by most publications however there is no consistent approach to bleeding classification. Bleeding rates were shared in 35 studies. The overall reported mean was 12.3% (SD 11.3, 95% CI 8.4-16.2) while the median was 12% (IQR 3.8-18.4%).
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<td>27 (7.5)</td>
<td>n/r</td>
</tr>
<tr>
<td>Aburto (133)</td>
<td>n/r</td>
<td>n/r</td>
<td>5 (1.9)</td>
<td>n/r</td>
<td>1 (0.38)</td>
</tr>
<tr>
<td>Troy (103)</td>
<td>59/65</td>
<td>14/12</td>
<td>59/65</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Koslow (134)</td>
<td>66/120</td>
<td>13/12</td>
<td>66/120</td>
<td>6 (5)</td>
<td>1 (0.09)</td>
</tr>
<tr>
<td></td>
<td>Diagnostic Yield</td>
<td>Complication Rates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------------</td>
<td>--------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td><strong>Gnass (135)</strong></td>
<td>90/114 (78.9)</td>
<td>n/r</td>
<td>5 (4.4)</td>
<td>5 (4.4)</td>
<td>n/r</td>
</tr>
<tr>
<td><strong>Zhou(136)</strong></td>
<td>134/155 (86.5)</td>
<td>19 (12.3)</td>
<td>3 (1.9)</td>
<td></td>
<td>n/r</td>
</tr>
<tr>
<td><strong>She(137)</strong></td>
<td>80/121 (66.1)</td>
<td>16 (13.2)</td>
<td>18 (14.9)</td>
<td>13 (9.3)</td>
<td>1 (0.8) *</td>
</tr>
<tr>
<td><strong>Pajares(138)</strong></td>
<td>59/124 (47.6)</td>
<td>9 (7.3)</td>
<td>3 (2.4)</td>
<td>2 (1.6)</td>
<td>n/r</td>
</tr>
<tr>
<td><strong>Inomata(139)</strong></td>
<td>75/87 (86.2)</td>
<td>16 (18.4)</td>
<td>4 (4.6)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Wang(140)</strong></td>
<td>48/70 (68.6)</td>
<td>4 (5.7)</td>
<td>3 (4.25)</td>
<td>3 (4.25)</td>
<td>n/r</td>
</tr>
<tr>
<td><strong>Hetzel(141)</strong></td>
<td>n/r</td>
<td>20 (15.6)</td>
<td>21 (16.4)</td>
<td>11 (8.6)</td>
<td>n/r</td>
</tr>
</tbody>
</table>

Table 4.2: TBCB diagnostic yield and complication rates

* Reported as unrelated to procedure
4.4 DISCUSSION

Diagnostic yield

The evidence base surrounding the use of TBCB in the diagnostic work up has rapidly expanded over the last few years. Nonetheless it remains unclear which routine role TBCB should play in the work up of ILD patients.

As with diagnostic yield reporting for SLB studies, there are inconsistencies with some studies reporting definite pathological diagnosis while others report overall diagnostic yield which takes into consideration the overall clinical and radiological picture. Griff et al. (111) illustrates some of the pitfalls of diagnostic yield reporting. This case series retrospectively analysed the diagnostic yield of TBCB performed in 52 patients. The paper compared final MDT diagnosis with pathological diagnosis and found a 79% (41/52 cases) correlation between the two over a wide range of ILDs. In 21 cases there was no correlation between histopathology and final MDT diagnosis. In 4 cases no specimens containing alveolar tissue were available but mucosal tissue in the specimens showed granulomas consistent with sarcoidosis and hence histopathological diagnosis was reached despite this. This demonstrates that even biopsies that could easily be classed as none-diagnostic (as not to biopsy the target parenchymal tissue) can contribute to an overall diagnosis. Whether or not such a specimen should be classified as a positive diagnostic sample of TBCB depends on the definitions used. The authors reported the highest diagnostic yields in disease processes that manifest broncho centric changes like sarcoidosis (yield of 83%), COP (89%), and hypersensitivity pneumonia (HP, 86%). They report a 10/15 (67%) histopathological to clinical diagnosis concordance for UIP pattern (both IPF and scleroderma associated ILD with UIP pattern) with 14 of these cases having a HRCT
pattern of possible or probable UIP pattern and one (7%) inconsistent with UIP pattern. Interestingly, the final MDT diagnosis was in part based on available histopathology and the authors failed to report whether histopathology changed MDT diagnosis for any cases. While this is not reported one must assume that the “inconsistent with UIP” pattern on HRCT was re-classified as UIP based on histopathology. This approach can conflate diagnostic yield of biopsy with diagnostic concordance. Overall, Griff et al show up the many potential pitfalls of comparing reported diagnostic yield between studies as the yield is dependent on clear definition what constitutes a diagnostic sample, which comparator is used as well as case mix.

Lentz et al reported a case series of 104 consecutive patients with diffuse parenchymal lung disease (DPLD) who underwent TBCB. They only achieved a confident histopathology diagnosis in 44.2%, but this increased to 68.3 % at MDT discussion and nonetheless TBCB changed management in 70.2% (124). While their methodology was improved compared with the previous paper discussed it still has several drawbacks. The confidence of histopathological and MDT diagnosis as well as treatment changes were independently scored by two clinicians with ILD expertise. This was done retrospectively and unblinded through case record reviews. A histopathologic diagnosis was deemed “confident” if the pathologist identified a specific disease process or strongly favoured one disease over, at most, one additional consideration. A confident MDT diagnosis was counted when the two reviewers independently agreed that the MDT diagnosis was reached with a high degree of certainty and no contradictory data were obtained during the clinical follow-up on retrospective review of medical records. What constitutes a high degree of certainty, or any objective scoring measure was not described. Treatment change attributed to TBCB was again determined through retrospective review of records by
the two ILD specialists. The lack of blinding and objective scoring measures decreases the otherwise valuable approach of including histopathological, MDT and treatment outcomes in the authors assessment of TBCB utility.

Hagmeyer et al (143) analysed diagnostic yield and pathological inter-observer agreement in 32 patients. The inter-observer agreement between pathologists was good with a kappa value of 0.8. In 23 out of 32 cases (72%) the pathological diagnosis correlated with the clinical diagnosis and was therefore considered “definite”. Eight of the nine remaining patients underwent SLB which led to a definite diagnosis in 6 cases (75%). Pathological findings from TBCB showed a good congruence with the SLB in seven of the eight SLB subjects (88%). In these seven cases, SLB confirmed the suspected histological results obtained from TBCB. This was a methodological sound study with a small sample size its main drawback.

A recent prospective, pan-European study (141) of 128 patients with IIP has demonstrated that TBCB significantly increases MDT diagnostic confidence. A central research MDT panel assigned their level of confidence for the first-choice diagnoses in four steps: 1. clinicoradiological data alone, 2. addition of BAL findings, 3. addition of TBCB and 4. SLB findings (if available). The proportion of confident diagnoses (likelihood ≥ 90%) increased from 22.7% after BAL to 53.9% after TBCB and when provisional diagnoses with high confidence (likelihood ≥70%) were included this increased further from 60.2% to 81.2%. SLB was performed following local discussion in nine of 128 cases (7.0%). In 6 cases SLB confirmed the TBCB diagnosis, in 2 cases the first line diagnosis changed and 1 case remained unclassifiable. This study elegantly confirmed that the addition of histopathology obtained through TBCB significantly increases the confidence in a MDT diagnosis, a phenomenon previously
described in the seminal work of Flaherty et al (34) for SLB histopathology. In their study, histology had the largest influence on final diagnosis in MDT discussion.

Similarly, Tomassetti et al (106) retrospectively evaluated 426 cases (266 TBCB, 160 SLB) in a step-wise MDT approach first pioneered by Flaherty. A comparable degree of final MDT consensus diagnosis was demonstrated with either TBCB or SLB (29% to 63% and 30% to 65% respectively). They were able to show that an IPF diagnosis reached based on TBCB is strongly predictive of survival, thereby providing important prognostic, as well as diagnostic, information.

Less encouragingly, Romagnoli et al (104) published a small, prospective trial of 21 patients who underwent TBCB in 2 separate lobes followed by immediate VATS-SLB of the same lobes. All samples were reviewed by a single pathologist who found poor concordance between the two modalities with agreement in only 38% of cases (8/21), k-concordance of 0.22. Compared with final local MDT diagnosis TBCB agreed in 48% compared to SLB 62% agreement. However, the study was criticised for its poor methodology and small sample size.

In 2016, Ravaglia and colleagues(89) also set out to retrospectively compare the diagnostic yield between SLB (150 cases) and TBCB (297 cases). Histopathological diagnosis was reached in 82.8% of patients with TBCB and 98.7% after SLB (P=0.013), though again the diagnosis was based entirely on histopathology and not consensus MDT.

Therefore, the jury was still out as to whether TBCB was inferior, superior or equal in its utility compared with SLB.
Late 2019 saw the long-awaited announcement of the results from the Australian COLDICE study (103), a multicentre study comparing TBCB and SLB head-to-head by acquiring biopsies in the same sitting, from the same lung lobe. 65 patients had a total of 130 biopsies taken. The two primary endpoints were histologic agreement between TBCB and SLB and agreement on final consensus MDT diagnosis for matched specimens. COLDICE was methodologically rigours: sample size was adequately powered; patient selection and procedures were standardised. Three blinded pathologists assessed the randomly assigned and deidentified TBCB and SLB samples, first individually and then together to reach a consensus agreement. The by now familiar stepwise MDT process followed with histopathology presented last. Panel members then recorded their confidence of diagnosis as low (51–60%), high (70–89%), or definite (90–100%). Histopathological concordance between TBCB and SLB was 70·8% (kappa value 0·70, 95% CI 0·55–0·86) and diagnostic agreement at multidisciplinary discussion 76·9% (0·62, 0·47–0·78). As in previous studies, COLDICE again demonstrated that the addition of histopathology to clinical-radiographic information impacted the diagnosis at MDT in 74% (48/65) TBCB specimens and 77% (50/65) SLB specimens. Twenty-six TBCB specimens were deemed unclassifiable or low confidence by MDT diagnosis. Of these, 23% (6/23) were reclassified into high or definite by SLB but in the remaining 77% (20/26), SLB offered no additional diagnostic confidence. This again highlights the intricacy of diagnosis in ILD and the reliance on MDT to reach consensus opinion.

Complications
Moderate to severe bleeding occurred in 384 out of 3325 cases (11.5%). The subclassification or grading of bleeding into minor, moderate or major bleeding is undertaken by most publications however there is no consistent approach to bleeding classification. The British Thoracic Society classifies bleeding as shown in Table 4.3.

<table>
<thead>
<tr>
<th>No bleeding</th>
<th>Traces of blood with no need for continuous suctioning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bleeding stops spontaneously</td>
</tr>
<tr>
<td>Mild bleeding</td>
<td>Continued suctioning of blood from the airways.</td>
</tr>
<tr>
<td></td>
<td>Bleeding stops spontaneously</td>
</tr>
<tr>
<td>Moderate bleeding</td>
<td>Intubation of the biopsied segment with the bronchoscope</td>
</tr>
<tr>
<td></td>
<td>into the wedge position</td>
</tr>
<tr>
<td></td>
<td>Use of adrenaline or cold saline to stop bleeding</td>
</tr>
<tr>
<td>Sever bleeding</td>
<td>Placement of bronchus blocker or catheter, applying fibrin</td>
</tr>
<tr>
<td></td>
<td>sealant</td>
</tr>
<tr>
<td></td>
<td>Resuscitation, blood transfusion, admission to critical care</td>
</tr>
<tr>
<td></td>
<td>unit or death</td>
</tr>
</tbody>
</table>

Table 4.3 BTS Classification of bleeding severity during bronchoscopy(144)
This classification is not easily applied to TBCB due to the routine use of endobronchial adrenaline and endobronchial blockers by most groups. The bleeding risk during TBCB is undoubtedly serious and had been examined in a prospective, randomized, multicentre cross-over trial by Hetzel et al (132) of 359 patients against standard transbronchial forceps biopsies (TBB). Overall bleeding was significantly greater in TBCB versus TBB with 72.7% and 48.2% respectively (p <0.001) and what has been termed “clinically relevant” bleeding (i.e., moderate to severe bleeding) was also significantly higher in TBCB (16.2% vs. 4.2%, p < 0.05). There have been no recorded fatalities secondary to massive haemorrhage in the literature though cases requiring intubation and ventilation do exist (126). Recognising the potentially fatal complication of endobronchial bleeding has led to widespread adoption of routine use of endobronchial blockers as well as other measures. Hagmeyer et al report the significant reduction of moderate to severe bleeding events from 80% to 0% following procedure safety adaptations. (131) It is now recommended by both the CHEST guidelines and consensus report on TBCB from 2019 (145) and in the expert statement from the Cryobiopsy Working Group on Safety and Utility from 2018 (146) that endobronchial blockers should be used in all cases and procedures performed either with rigid bronchoscopy or endotracheal tube in place.
Pneumothoraces occurred in 371 out of 3700 procedures (10.0%). There is some evidence that the pneumothorax rate is increased in fibrotic ILDs. Ravaglia et al (101) report a pneumothorax rate of 20% in their case series of 241 patients, but this rises to 28% in patients with fibrotic ILD. Conversely, Gershman et al (147) found no significant difference in the pneumothorax rate between lung transplant patients, immunocompromised patients and patients with suspected DPLD. Their overall pneumothorax rate was 5% in a case series of 300 procedures. However, the diagnostic breakdown of their DPLD cohort was not reported, and the relatively low pneumothorax rate compared to the Ravaglia series is likely secondary to a lower number of patients with fibrotic ILD.

Ravaglia et al (101) also compared outcomes of patients undergoing SLB and TBCB in their centre over a 10 year period. 155 patients underwent SLB and 241 TBCB. The mean age of the patients was similar in the two groups (57.74 ± 11.29 years in SLB group vs 56.70 ± 11.29 years in TBCB group). Mortality and complications were evaluated at 30 and 90 days. In the SLB group mortality was observed in 3.9% of patients and minor complications were experienced in 10.6% of patients. In the TBCB group the most frequent complication was pneumothorax (19.9%) but only 20% of these required drainage with ICD.

4.5 Conclusion

TBCB is now well established in several specialist centres and large case series have shown it to be both safe and effective with mounting evidence that it provides valuable diagnostic information in the context of MDT work up in ILD patients. It could potentially replace SLB as a first line pathological diagnostic tool. However, the utility outside of
specialized centres with adequate case volumes to establish expertise is unknown limiting external validity of outcome data.
Chapter 5 Setting up a transbronchial cryobiopsy service for the diagnosis of ILD – The UCLH experience
5.1 Introduction

Endobronchial cryosurgery techniques have been used since the 1970s (148) and became firmly established in the bronchoscopy suite for endobronchial tumour debulking in the mid-nineties (149, 150). As outlined in Chapter 6 they were first used to acquire lung parenchyma via a transbronchial biopsy in 2009 by Dr Hetzel, based in Tuebigen, Germany (27). In 2013, the UCLH/UCL respiratory team set out to establish the first transbronchial cryobiopsy service to aid the diagnosis of ILD patients in the UK. A single manufacturer, ERBE Elektromedizin GmbH (Tuebigen, Germany), provides bronchoscopy cryo-equipment compromising of the Cryo-unit and flexible cryo-probe. ERBE flexible cryoprobes and associated systems received CE marking under the Medical Devices Directive 1993/42/EEC on 24 July 1998 (ERBOKRYO CA) as class IIb devices. Cryoprobes operate on the Joule-Thomson principle that expansion of a compressed gas leads to a cooling effect. Pressurised gas is channelled from bottled storage into the cryo-probe at the level of the cryo-unit and decompressed at the tip of the cryoprobe leading to rapid expansion and corresponding temperature drop. This causes freezing of the metal tip and adjacent tissue (see Figure 5.1a and 5.1b). ERBE cryo-units can be operated with either compressed nitrous oxide or carbon dioxide (151). Nitrous oxide is our preferred coolant leading to a maximal temperature low of -89°C, depending on the age of the cryoprobe used as its cooling efficiency deteriorates with wear and tear due to kinks in the metal tubing affecting gas flow.
Figure 5.1a: Cryoprobe schematic (adapted from Casoni et al (152))

Figure 5.1b: Cryoprobe (©TM)
5.2 Methods

As there were no UK centres performing TBCB biopsies in the UK I secured a two-week visiting fellowship in November 2013 with Dr Jurgen Hetzel in Tubingen to learn the technique before establishing it at UCLH. While there, I also visited the ERBE factory to familiarise myself with their research and development department. Following the necessary approvals from the New Interventional Procedures with the Clinical Effectiveness Steering Group and working with the interventional bronchoscopy team I elected to establish the new TBCB service using the ERBEcryo 1 (ERBE, CE 0124) unit run with nitrous oxide, and re-usable cryoprobes.

Only patients over 18 years old, with evidence of ILD on HRCT were considered for TBCB biopsy. All patients underwent clinical assessment by an ILD specialist and were discussed at either our ILD MDT (Multidisciplinary team meeting) or weekly respiratory radiology meeting. Only patients in whom considerable diagnostic doubt remained after thorough clinical work up were considered for TBCB. All patients were assessed by me regarding suitability for the procedure and to obtain informed consent. All except one acute patient had pulmonary function tests performed prior to bronchoscopy as well as a full blood count and clotting profile. Patients on anti-platelet or anticoagulant drugs had these stopped if clinically safe to do so. Patients who had a contraindication to stopping these medications were not put forward for TBCB. Selected patients underwent echocardiography to exclude pulmonary hypertension. Patients with an estimated Pulmonary Artery Systolic Pressure of more than 40mmHg were deemed to be too high risk for the procedure.
Patients requiring long-term oxygen therapy or ambulatory oxygen therapy were excluded.

We keep an emergency kit box in the bronchoscopy room compromising of a full chest drain kit, double lumen endotracheal tube and J-wire. Anaesthetists attending the list unfamiliar with the procedure are briefed on the bleeding risk and the emergency kit box is checked for completeness prior to starting the procedure.

Exact TBCB procedures and approach vary widely. We have therefore summarised our current Standard Operating Protocol:

**SOP for TBCB at UCLH**

Patients are admitted to the day surgery unit at UCLH. All procedures are carried out in the hybrid theatre.

Topical lidocaine is sprayed in the oropharynx and patients are pre-medicated with 1g of IV tranexamic acid, to minimise bleeding risk should no contraindications exist, to minimise bleeding risk. Patients are deeply sedated by a consultant anaesthetist using propofol and remifentanil, and occasionally midazolam (as per anaesthetic preference). Oxygen is insufflated continuously through nasal cannula; spontaneous breathing is maintained throughout the whole procedure. Oxygen saturation, blood pressure, ECG and transcutaneous carbon dioxide partial pressure are monitored continuously. Patients are supine on the image intensifier trolley to allow fluoroscopy guidance during the procedure. The patient is then intubated with an uncuffed,
re-enforced endotracheal tube (ET) (Bronchoflex, RUSCH) which possess an additional side port originally designed to administer oxygen (see Figure 5.2)

Figure 5.2: Uncuffed, re-enforced endotracheal tube (© Rusch Teleflex)

The bronchopulmonary segments for biopsy are determined prior to the procedure according to the HRCT scan. An interventional, flexible bronchoscope (1T, Olympus) is passed through the ET tube to the pre-selected broncho-pulmonary segment. A 5ml aliquot of 1:10000 dilution of adrenaline is administered to the chosen segment. Using the additional side-port on the endotracheal tube a Fogarty arterial embolectomy catheter (size 5F) is placed into the chosen segment as a prophylactic endobronchial blocker. It is inflated briefly to check positioning. (Figure 5.3)
Figure 5.3: Fogarty balloon used as endobronchial blocker (©TM)
Following this a flexible cryoprobe (ERBE, CE 0124) measuring 90cm in length and 2.4mm in diameter is passed through the bronchoscope. Under fluoroscopic guidance, the cryoprobe is navigated towards the selected area aiming to keep 10-15mm from the pleura. The probe is cooled with nitrous oxide so the temperature in the probe’s tip decreases to approximately -89°C for 3-5 seconds. Then the cryoprobe and bronchoscope are simultaneously retracted, retrieving the sample frozen to the tip of the probe. Simultaneously, the Fogarty balloon is inflated. The frozen specimens are thawed in saline and then fixed in formalin. The bronchoscope is then re-inserted to check for bleeding. If there is significant bleeding further adrenaline is instilled and the Fogarty balloon is kept in place as a tamponade till bleeding subsides. We aim to take 3-5 biopsies however the exact number is dependent on size of the obtained specimens and any complications arising.

At 2 hours after the procedure a chest X-ray is performed to exclude pneumothorax. If there are no complications the patients will be discharged home following their chest x-ray.

Histological specimens are processed in the department of pathology according to standard protocols with serial sectioning. Our respiratory pathologists with experience in ILD report all specimens with knowledge of the clinical scenario.

All patients are then re-discussed in the specialist ILD MDT to ascertain firstly a definitive pathological diagnosis and secondly a consensus MDT diagnosis.
Since establishment of the procedure, we have introduced several adaptations to the protocol which included the routine use of an ET tube, pre-medication with IV tranexamic acid and endobronchial adrenaline as well as placement of a Fogarty arterial embolectomy catheter (size 5F) as a prophylactic endobronchial blocker. All these adaptations were adopted to decrease complications and improve the safety profile.

We set out the below TBCB check list as an aide-memoir for staff involved:
Transbronchial cryobiopsy checklist

Before procedure:

All patients must be discussed in ILD MDT or if time limited in x-ray meeting.

All patients must be pre-assessed for suitability and to explain risks.

All patients: MRSA swabs, FBC, clotting, Group and Save

If worried about pulmonary hypertension they need an ECHO to exclude it; general cut off is not to biopsy if PAP >45mmHg.

Book procedure slot by email ThoracicProcedures@uclh.nhs.uk

Book radiographer by emailing imaging@ucl.nhs.uk

Put fluoroscopy booking request on system.

On the day:

Equipment:

- 90cm 2.4mm cryoprobe - (check it is freezing using sterile water before starting)
- Fogarty balloon (5Fr) + 2ml syringe + 3 way tap with long tubing.
- Bronchoflex Rusch ET tube
- Silcospray to lubricate ET tube and cryoprobe.
- Bowl of warm water and saline container inside – transfer samples to histopathology pot at end of procedure

Drugs: tranexamic acid 1g IV (anaesthetist to give at start of procedure)

1:10000 dilution of adrenaline (cold)
**Emergency box:**
Check this is complete and in the room: double lumen tube; J wire (brief the anaesthetist about bleeding risk); chest drain kit.

**Consent for:**
Pneumothorax, bleeding, damage to teeth, sore throat, infection/cough, failure of procedure and anaesthetic risk

**Research:**
All TBCB patients should be consented to Prof Porter’s “Mechanism of Lung Injury study”.

**Post procedure:**
CXR 2h after or if patient unwell earlier. If no pneumothorax and no other concerns can go home with advice regarding delayed pneumothorax. I always give them my number.
Patient to be rediscussed in MDT once results available. Mark histopathology form for FAO Dr Mary Falzon or Dr Elaine Borg.

**Patient Information Leaflet**
A patient information leaflet was specifically developed to provide procedure information for TBCB patients. (Appendix 10)
**Data collection, audit and clinical governance**

All patient data was collected prospectively to include baseline characteristics, procedure related data (number of biopsies taken), complications and histological outcome data.

In compliance with local governance procedures “cryoscopic airway extraction” and “cryoscopic transbronchial lung biopsies” were registered as New Interventional Procedures with the Clinical Effectiveness Steering Group (CESG) at UCLH in 2012. Relevant safety and efficiency data was presented in March 2016 to the CESG and TBCB was signed off as an established procedure at UCLH.

Data was analysed and presented using Prism 9 (GraphPad, San Diego 2020)
5.3 RESULTS

We performed 36 procedures between November 2013 and November 2018 on 35 patients: 13 female and 22 male. Mean age was 62.4 years (SD 9.8), with no statistically significant difference between mean male age (61.8, SD 11.6) versus mean female age (63.5, SD 6.1) and identical median age of 65. (Figure 7.4)

![Age distribution](image)

Figure 5.4: Age distribution by gender

Mean FEV1 was 81.2% predicted (SD 19.4); mean FVC was 81.7% predicted (SD 19.3) and mean TLCO was 53.4% (SD 11.2) (Figure 5.5). Two patients were unable to perform gas transfer manoeuvres, one was not attempted due to low lung volume and for three gas transfer was not available.
A mean of 2.9 (SD 1.3) biopsies were taken per procedure (range 1-5) with a mean aggregate size of 428.8 mm$^3$ (SD 524.1) with the maximum recorded aggregate size 2000 mm$^3$ and the smallest 54 mm$^3$. Unfortunately, ULCH pathology services were outsourced in 2018 and specimen size stopped to be reported routinely and is therefore unavailable for 8 patients.

A firm pathological diagnosis was established in 23 patients (63.9%); non-specific changes were seen in 8 patients (three non-specific interstitial fibrosis and 5 non-specific inflammations with or without associated fibrosis). Three biopsies were reported as inconclusive. Two biopsies demonstrated normal lung parenchyma only and were therefore non diagnostic (Table 5.1).
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=36</td>
</tr>
<tr>
<td><strong>Diagnostic</strong></td>
<td></td>
</tr>
<tr>
<td>UIP</td>
<td>8 (22.2)</td>
</tr>
<tr>
<td>NSIP</td>
<td>5 (13.9)</td>
</tr>
<tr>
<td>Organising pneumonia</td>
<td>3 (8.3)</td>
</tr>
<tr>
<td>Hypersensitivity Pneumonitis</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Possible UIP</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Fibrotic NSIP</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>CLL</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>Emphysema and fibrosis</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>Non-specific inflammation +/- fibrosis</td>
<td>5 (14)</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>3 (8.3)</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>3 (8.3)</td>
</tr>
<tr>
<td>Non-diagnostic (normal parenchyma)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td><strong>Non-diagnostic</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=13 (36.1%)</td>
</tr>
</tbody>
</table>

Table 5.1: TBCB Histopathological diagnostic yield

15 patients experienced complications (40.5%):

one major and three moderate procedure associated bleeding events (n=4, 10.8%) requiring prolonged endobronchial suctioning and tamponade of the
affected segments; one of the four patients was admitted overnight for observation, the other three were discharged the same day.

10 patients suffered a pneumothorax (27%); of those three did not require any intervention, while 7 (18.9%) required placement of Seldinger chest tubes (one was a delayed presentation to a different hospital). All lungs re-expanded without the need for surgical intervention.

One patient (2.7%) was admitted following the procedure as feeling generally unwell with headache and malaise, though had normal vital observation, chest imaging and laboratory tests and was subsequently discharged.

There were no recorded exacerbations and no deaths.

Of interest, 8 out of the 10 patients experiencing a pneumothorax had a diagnostic biopsy (80%) in contrast to only 1 out of the 4 with major or moderate bleeding event (25%).

One patient was subsequently referred for surgical lung biopsy.

Patient 7 underwent traditional transbronchial forceps biopsy prior to TBCB, allowing direct comparison of the two modalities (Figure 5.6a and 5.6b). This demonstrates not only the marked size difference between the two biopsy techniques but also illustrates crush and haemorrhage artefacts in the forceps biopsy and preserved architecture in the cryo biopsy (Figure 5.7).
Figure 5.6a Cryo Biopsy (x20)
Architecture Preserved
Total biopsy area 46.81mm²
Mean biopsy area 11.7mm²

Figure 5.6b Forceps Biopsy (x20)
Crush and haemorrhage artefact
Total biopsy area 14.11mm²
Mean biopsy area 2.8mm²
Figure 5.7: Haemorrhage artefact in forceps biopsy (x20)
5.4 DISCUSSION

While the high overall complication rate of 40.5% coupled with a histopathological yield of 63.9% is disappointing these results are not particular outliers compared with other published smaller case series.

In our case series 10 patients suffered a pneumothorax (27%). The literature review in chapter 4 found a pooled pneumothoraces rate of 10.0% (371 out of 3700 procedures). A pneumothorax rate of around 20% has been published by several groups, for example Kronborg-White (26%) (120), Ramaswamy (20%) (116) or Ravaglia (20%) (89). Subsequently, Ravaglia et al (102) published the largest case series yet of 699 patients in which a pneumothorax rate of 13.4% was described. I have identified several contributing factors to our relatively high pneumothorax rate. One is the procedural learning curve – our relatively small case series did not allow us to reach the plateau of expertise. Secondly, pneumothorax rates are increased in patients with a fibrotic ILD (152) and we pre-selected our patients for mostly that phenotype.

Lastly we undertook all but 3 biopsies with a 2.4mm cryoprobe; latest recommendations are to use a 1.9mm probe (145). However, the upside to causing a pneumothorax is a higher diagnostic yield as 8 out of our 10 pneumothorax patients had diagnostic biopsies taken. As UIP changes in IPF are, at least initially, found subpleural biopsies that have clearly come from that location will have a higher chance of being conclusive. Furthermore, when reporting surgical lung biopsy lung pathologists usually orientate themselves with regards to the orientation of the pleural surface. Hence inclusion of pleura in the specimen likely increased diagnostic confidence of our pathologists.
While any complication is disappointing it is worthwhile to bear in mind the complication rates of other lung biopsy modalities: for a traditional VATS biopsy it is per definition 100% and a recently published meta-analysis of CT guided needle biopsies for small lung nodules reported a pooled pneumothorax rate of 19%(153). The main worry for both procedures are prolonged air leaks, i.e., the lung not re-expanding. We reported no prolonged air leaks in our series.

The severe bleeding rate (1 patient, 2.7%) is in keeping with larger case series and led to the introduction of the routine use of an endobronchial blocker and pre-medication with IV tranexamic acid and endobronchial adrenaline with no further cases. 3 patients had moderate bleeding. The overall diagnostic yield for patients with severe or moderate bleeding was only 25% (1 in 4). This is in part because these procedures were abandoned after the bleeding even occurred (though mean specimen number was not significantly higher in other procedures) but also reflects the fact that bleeding is much more likely when the cryoprobe is placed off target, meaning too proximally.

The interplay of these two major complications – pneumothorax and bleeding – illustrates the concept of the TBCB “sweet spot”. For the highest diagnostic yield, one must target the sub-pleural region. Staying too proximal in an attempt to avoid this particular complication leads to a lower diagnostic yield (146) and an increase of bleeding as blood vessels are larger and the arteries lack the protection of complete cartilage shields that are found in the more central airways(154). Therefore, one might argue that we should be less worried about high pneumothorax rates and more about severe bleeding.
Our reported histological diagnostic yield appears unsatisfactory at first glance, however again when compared to the literature, and taking different reporting of histological findings into account, it does compare passably. Reported diagnostic yield of TBCB ranges from 51% to more than 90%, with an average of 80% (89, 102, 111, 117, 119, 120, 128, 152, 155, 156, 157). However, some studies are less rigorous in their classification of what qualifies as diagnostic with some including “interstitial fibrosis” as a diagnosis. Only 3 (8.3%) of our samples were truly undiagnostic in the sense of not providing enough alveolar tissue for examination. We further classified as non-diagnostic the 2 cases only containing normal lung tissue and the 8 cases with non-specific findings (non-specific inflammation and or fibrosis, interstitial fibrosis). There are several factors that might have influenced the low diagnostic yield. Firstly, our patient selection was such, that only the most challenging cases were put forward for biopsy. With reduced pre-test probability any histological diagnosis will be more challenging (i.e., if there is an UIP pattern visible on HRCT it is more likely to be found on biopsy as well). We do know that about 5% of surgical lung biopsies are truly unclassifiable (158) and that TBCB has a lower yield than SLB due to size, lack of spatial orientation for the histopathologist and general less familiarity with this new sampling technique. This was also thought to be another factor in our poor histology outcome. The respiratory histopathology team changed its composition and working patterns around 2016/2017 as part of the outsourcing of histopathology services. Our histopathologist stopped attending the ILD MDT and was therefore reporting in isolation. An increased number of biopsies were being sent to an external centre for second reviews as pathology was struggling. The learning curve for
histopathologists being faced with a new biopsy technique is as real as that of the bronchoscopist and frequent changes in team composition are unhelpful. We performed 37 procedures over the course of five years which is a relatively low volume compared to the large continental European case series and undoubtedly this hampers the learning curve of all involved. In the UK context of minimal utilisation of SLB in the diagnostic work up of ILD it is a relatively large number for a single centre. Prior to the introduction of TBCB at UCLH 1-3 patients were referred for surgical diagnostics/ year. Patient numbers are also restricted since IIPs remain relatively rare diseases.

While TBCB is becoming more established in continental Europe it has only been taken up by one other UK centre. One of the main reasons for this is that propofol sedation for bronchoscopy is widespread in Europe but not routinely used in the UK. Propofol is administered by pulmonologists themselves in some settings in continental Europe but requires an anaesthetist in a UK setting. We have succeeded in establishing TBCB at UCLH in part due to an already well-established interventional bronchoscopy service that routinely provides regular propofol lists. We made 10 UIP/ probable UIP diagnosis therefore opening the door to antifibrotic treatment to patients who would have otherwise not qualified and might have required a potentially more harmful SLB. We also diagnosed one case of pulmonary CLL involvement. The literature repeatedly describes the important diagnosis of ILD mimics in the form of malignancies such as mucinous adenocarcinoma or fungal infections by Lentz et al (124). In the largest series of 699 patients by Revaglia et al 47 patients (6.7%) were diagnosed with a
malignancy, 14 lymphoproliferative disorders and 33 epithelial neoplasms (102). In fact, malignancy was the third commonest diagnosis after UIP pattern and OP in that case series which should act as a warning against diagnostic complacency in the light of changing requirements for access to antifibrotic therapy.

**Limitations**

This case series is limited by the low patient volumes and non-dedicated ILD histopathology service. This led to change of reporting standards (for example not including specimen size) and MDT team composition which increased the difficulty in comparing histopathology diagnostic yield within the series. We plan to have all our TBCB specimens reviewed by an external pathology team with TBCB ILD expertise.
5.5 CONCLUSION

This chapter sets out the steps taken to set up a new interventional bronchoscopy service at UCLH and the safety features introduced to avoid major complications.

Our complication rate is comparable - though at the upper end of the spectrum - with that of major case series as we select our patients from a patient pool with a likely fibrotic ILD. Our diagnostic rate is below most reported case series which could be explained by our and our pathologists learning curve and a pre-selection of the most challenging diagnostic cases as well as rigorous classification of what constitutes a diagnostic sample.

The long-term need for any invasive biopsy techniques is likely diminishing but there will be a subset of patients that continue to require histopathological certainty. It remains unclear if TBCB or novel VATS techniques such as awake VATS will be the best way forward.
Chapter 6

INHALE TRIAL-

Study to assess inhaled drug distribution in the distal lung and interstitium using cryobiopsy samples from subjects with suspected Interstitial Lung Disease undergoing cryobiopsy for clinical reasons.
6.1 INTRODUCTION

The interstitial lung diseases (ILDs) are a group of over 200 lung disorders that are characterised by interstitial fibrosis, and lead to declining lung function, respiratory failure and ultimately death. The most severe fibrotic (f)ILD is Idiopathic Pulmonary Fibrosis (IPF).

Two oral drugs, pirfenidone and nintedanib, are licensed for the treatment of IPF and have now been shown to have benefits in other fILDs (94) but both have limiting adverse effects. Inhaled therapy for ILD offers the advantage of drug delivery direct to the lung, thereby minimising systemic exposure and associated side effects. However, lung deposition, absorption and local therapeutic response may be altered in the fibrotic lung (159).

Assessment of lung drug levels using bronchoscopic lavage has become a critical component of inhaled drug development but lacks spatial information of the site or region of deposition. The advent of transbronchial cryobiopsy (TBCB) to sample the lung parenchyma for diagnosis of ILD allows access to important histological information using a minimally invasive bronchoscopic technique (103). TBCB potentially also allows more rapid lung tissue sampling following drug inhalation, compared to traditional surgical lung biopsies therefore shortening the time during which the inhaled drug can be cleared from the lung before analysis. Furthermore, participants are not subject to mechanical ventilation which could theoretically alter inhaled drug distribution in surgical participants.

Liquid Chromatography - tandem Mass Spectrometry (LC-MS/MS) is traditionally used for the analysis of homogenised tissue samples and therefore any spatial information regarding drug distribution within the tissue is
lost. In contrast, Matrix Assisted Laser Desorption Ionisation – Mass Spectrometry (MALDI-MS) imaging allows detection and characterisation of molecules from tissue (160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171) and supports the spatial visualisation of drug distribution in tissue samples. Analysing the same TBCB biopsy with a combination of LC-MS/MS, MALDI-MS imaging and histopathology can therefore allow minimally invasive assessment of drug distribution within the diseased fibrotic lung. Fehniger et al (171) have previously demonstrated inhaled drug distribution of ipratropium using MALDI-MS imaging in the proximal airways of patients with suspected airway obstruction or tumours. Ipratropium readily ionises and the MS/MS fragmentation pattern produces two major fragment ions (at m/z 166.0 and 123.9) that can be detected using MALDI-MS.

In this clinical study, based on work from pre-clinical enabling study, we combined, for the first time, rapid distal sample acquisition using TBCB with the mass spectrometry modalities of LC-MS/MS and MALDI-MS imaging, together with histopathology to demonstrate inhaled drug delivery to fibrotic, distal human lung parenchyma in participants with diagnosed ILDs. Whilst this study was designed as a proof of concept with a low participant number (n=5), we can present confirmation that inhaled drug therapy is a feasible route of administration for fibrotic ILD, which could avoid the significant systemic side effects of current oral therapy. To our knowledge this is also the first time that TBCB has been used in translational research. Our work was based on the findings of a scaled preclinical study carried out by the GSK team that was initially conducted in rats to assess whether
Ipratropium can be detected in distal lung and over which time-course. A scaled dose of ipratropium bromide, equivalent to the clinical dose, was nebulised to male Wistar rats. Terminal lung samples were taken at varying time points up to 65 mins post-dose and 5 mm ex-vivo biopsies were embedded into material suitable for MALDI-MS imaging. The results from this study were used to optimise assay conditions, due to the anticipated challenges with respect to the detection of a single clinical dose of ipratropium in relatively small lung biopsy samples. The preclinical study allowed sample handling methods and detection limits of ipratropium in rat lung samples (similar in size to human cryobiopsy samples) to be assessed by LC-MS/MS and MALDI-MS imaging. Widespread and even distribution of ipratropium was observed in both rat lung sections and equivalent sized biopsies to those expected from TBCB.

The MALDI-MS imaging experiments involve the ionization of molecules in a raster from sections of biopsies placed on conductive glass slides using a pulsed laser. The resulting gas-phase analyte ions are separated according to their mass to charge ratio ($m/z$) by a mass analyser. The addition of an organic matrix to the surface of the sample section is used to increase ionisation efficiency and for the extraction of analytes from the tissue sample. The focus of MALDI-MS imaging is to obtain chemical information (in this instance the detection of ipratropium) from a tissue section with the highest spatial resolution and at biological relevant sensitivities, without compromising the original spatial distributions of the drug(s)/components of interest. The spatial resolution achieved by MALDI-MS imaging is limited by the size of the matrix
crystal deposited on the sample section and the laser spot size of the instrument.

Ipratropium was selected for this study as it readily ionises, and the MS/MS fragmentation pattern produces two major fragment ions (at m/z 166.0 and 123.9; Figure 6.1) that can be detected using MALDI-MS. Ipratropium bromide was selected for this study as it readily ionises. The limits of detection (LOD) for ipratropium in the MALDI-MS imaging experiments was previously determined as 1 pg/µL spotted onto a rat lung tissue section. For LC-MS/MS, the lower limit of quantification (LLOQ) measured for ipratropium in the non-clinical study was 2 pg/rat lung section and 1 pg/5 biopsy sections.

For the avoidance of doubt, I would like to re-state my contributions to this clinical trial and which elements were delivered by my collaborators at GSK (please also see chapter 1, section 1.4). I had key involvement in developing the protocol for the INHALE trial and delivered the protocol, wrote study documents and conducted all relevant information and data governance compliance activities (for example sponsor study site visits). I arranged relevant contracts and data sharing agreements. I was solely responsible for screening, recruitment, consent and assessment of study participants. I carried out the study procedure with support of the clinical team in the bronchoscopy suite and my interventional bronchoscopy colleague Dr Ricky Thakrar. I was responsible for initial processing and correct shipping of research samples. I was responsible for carrying out most of the data collection, supported by our research nurses Therese Bidder and Geeta Vekaria.
I had some involvement in the sample processing for MALDI MS analysis, but most of the sample processing and data analysis was carried out by Dr Peter Marshall and his team at GSK, Stevenage. Prof Porter and I were part of the team interpreting the gained data, specifically advising on histology images and potential co-location with compound to fibrotic area. The resulting research publication was again a collaborative effort in which I focused on the clinical trial methods and presentation, interpretation and discussion of results.

Figure 6.1 Ipratropium (structure and fragmentation)
6.2 AIMS

This was a single centre, prospective study to evaluate the ability of inhaled Ipratropium bromide to reach the distal lung by processing Transbronchial cryobiopsy (TBCB) and endobronchial forceps biopsy samples using the mass spectrometry techniques of Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS) and Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS) as well as histology techniques.

Primary Objective

- To evaluate the ability of inhaled Ipratropium bromide to reach the distal lung in suspected ILD patients, by analysing transbronchial cryobiopsies from fibrotic regions of the lung.

Secondary Objectives

- To assess Ipratropium bromide distribution in endobronchial forceps biopsy samples
- To compare the distribution of inhaled Ipratropium bromide in proximal and distal lung

Primary Endpoints

- Images and data generated using mass spectrometric techniques and histology showing the distribution of Ipratropium bromide within the cryobiopsy samples taken from participants with suspected ILD.

Secondary Endpoints
• Images and data generated using mass spectrometric techniques and histology showing the distribution of Ipratropium bromide within the endobronchial samples taken from participants with suspected ILD.

• Mass spectrometry and histology data showing distribution of inhaled Ipratropium bromide in the proximal and distal lung.
6.3 METHODS – PART A: Clinical STUDY

CLINICAL STUDY DESIGN

This was a single centre prospective interventional study to evaluate the ability of inhaled Ipratropium bromide to reach the distal lung by processing TBCB, and central airways by endobronchial biopsy samples, using mass spectrometry and histology techniques.

The study was not a clinical trial of a medicinal product, and the participants were undergoing TBCB for clinical reasons as part of their diagnostic pathway. Participants were administered study treatment (ipratropium bromide) to allow assessment of deposition of the drug in their lungs by use of TBCB and endobronchial biopsy.

Participants were not required to specifically attend hospital for additional study visits outside their usual clinical care. Study specific follow-up occurred 7 days after the procedure via a telephone call.

All participants were administered nebulised ipratropium bromide and underwent the TBCB procedure as part of their clinical diagnostic testing.

Study procedures are detailed further below.

No planned interim analysis was performed but data was reviewed in-stream as it became available.
STUDY PATIENTS

Participants over the age of 18 with suspected ILD and requiring TBCB for further diagnostic assessment, as determined by the ILD multidisciplinary team, were eligible to participate.

Inclusion criteria:

• 18 and above years of age inclusive, at the time of signing the informed consent.

• Participants with suspected ILD listed for TBCB for clinical reasons following review by the ILD services at University College London Hospitals in whom diagnosis has remained unclear following radiological and clinical assessment.

• Capable of giving signed informed consent

Exclusion criteria:

• Participants who have a known drug allergy or other contra-indication to Ipratropium bromide

• Known hypersensitivity to atropine or ipratropium bromide or any other known drug allergies that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.

• As a result of the medical history, physical examination or screening investigations, the physician responsible considers the participant unfit for the study.

• The participant is unable or unwilling to perform study assessments and procedures correctly.
• Participants with a recognised co-existing respiratory disorder (other than ILD) that in the opinion of the investigator would confound the study outcomes.

STUDY PROCEDURES

Patients were screened and consented to the clinical trial up to 6 weeks prior to biopsy procedure. Screening involved clinical assessment for fitness to participate, performance of spirometry (FVC and FEV1), physical examination and recording of demographic parameters.

All procedures were carried out in the Hybrid Operating Theatre - a combined operating theatre and interventional radiology suite situated in the day surgery unit at UCLH.

All participants received a single dose of 500 mcg nebulised ipratropium bromide via Phillips Porta Neb device (Amsterdam, Netherlands) with a SideStream aerosolising chamber (Respironics, Tangmere, UK) immediately prior to the start of bronchoscopy and before any sedation was administered.

Patients were sitting erect during drug nebulisation to allow optimal drug distribution.

As recommended by the European Respiratory Society guidelines on the use of nebulisers (172), the nebuliser was run until the low volume in the nebulising chamber caused spluttering or 10 minutes was completed, whichever occurred earlier. The typical volume left was 0.5 mL to 1.0 mL. It is not recommended to run a nebuliser to dryness.
TBCBs for diagnosis were performed as per standard local procedure (see Chapter 5 for details) using an ERBECRYO 1 unit with a 2.4 mm cryoprobe (ERBE, Tübingen, Germany).

Samples were taken in radiologically pre-selected fibrotic areas by passing the cryoprobe through a terminal bronchus to reach lung parenchyma approximately 1 cm from the pleural edge. Clinical samples were taken first. Next, one to two additional TBCB research samples were taken if safe to do so. Up to three endobronchial forceps biopsy samples were taken at the level of the right secondary carina as positive controls and to allow a comparison of proximal and distal drug deposition. The bronchoscopist determined the exact number of biopsies taken according to participant safety and the quality of the obtained specimens. Each study sample was individually embedded in cold (~4 °C) Poly[N-(2-hydroxypropyl)-methacrylamide] (pHPMA) immediately after collection(173), frozen on dry ice and stored at -80°C.

As per standard clinical practice, participants were monitored for safety continuously during the procedure and had a chest X-Ray and clinical review approximately 2 hours after the biopsy to exclude a pneumothorax. They had a study telephone consultation 7-14 days post procedure to assess for late adverse events and if present they were followed-up till until said the adverse event had resolved.

See Table 6.1 for a summary of the study schedule:
# Study Assessments and Procedures

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Screening&lt;sup&gt;1&lt;/sup&gt; (up to 6 weeks prior to the day of procedure)</th>
<th>Treatment (hours)</th>
<th>Period</th>
<th>Follow-up Phone Call (7-14 days post-procedure)</th>
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<tbody>
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<tr>
<td>Study Informed consent</td>
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<td>Inclusion and exclusion criteria</td>
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<tr>
<td>Demography</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Lung function (include Forced Vital Capacity [FVC] and Forced Expiratory Volume in 1 second [FEV&lt;sub&gt;1&lt;/sub&gt;])&lt;sup&gt;4&lt;/sup&gt;</td>
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<tr>
<td>Administer Ipratropium bromide</td>
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<td>X</td>
<td>←-----------------&gt;</td>
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</tr>
</tbody>
</table>

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1. Screening was conducted as part of standard of care for patient undergoing transbronchial cryobiopsy.
2. Procedure was conducted as part of standard of care and data was not collected in eCRF.
3. Past and current medication history up to 48 hours prior to transbronchial cryobiopsy were recorded.
4. If test otherwise performed within 3 months prior to first dose of study treatment, testing at screening was not required.
5. Observations taken on admission to endoscopy and then continuously from initiation of anaesthetic till early recovery as per standard of care.
6. Samples collected in up to 5 participants only.
7. X-Ray was performed at approximately 2 hours.
8. Participant were sent to recover in the designated recovery area as per local SOP.

Table 6.1: Study assessments and procedures
PATIENT CONSENT
Patients were given written information on the study (Participant Information Sheet – PIS) and consented to participate in the study following clinical consent was taken for the TBCB procedure.

WITHDRAWAL CRITERIA
Participants could withdraw from the study at any time at their own request or could be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons.

If a participant withdrew from the study, they could request destruction of any samples taken, and the investigator must document this in the site study records. The participants were made aware that this would not affect their future care. Participants were made aware (via the information sheet and consent form) that should they withdraw, the data collected may still be used in the final analysis.

ETHICAL APPROVALS AND FUNDING
The study protocol, any amendments, the informed consent, and other information that required pre-approval were reviewed and approved by London Camden and Kings Cross Research Ethics Committee (REC reference: 16/LO/2009) (See Appendix 4) in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP). The study was REC reference: 16/LO/2009). It was funded and sponsored by GlaxoSmithKline.
DATA COLLECTION and MANAGEMENT

Patient baseline demographic data, age, height and weight, lung function and concordant medication data was collected prior to procedure. Procedure specific data collected: date and exact time of ipratropium nebulisation (start and stop time), exact time of biopsy collection, anatomical site of biopsy collection, time when sample reached -80C freezer. Adverse event (AE) and serious adverse event (SAE) narrative data collection if required. Follow-up narrative data collection.

Participant data was entered into GSK defined eCRFs (Inform) and transmitted electronically to GSK. Verification of data accuracy and adherence to protocol requirements was achieved through regular monitoring visits at the investigational site. All investigators and responsible study staff attended a study site initiation meeting to review study protocol procedures, study requirements, and GCP responsibilities. Investigators and staff were given opportunity to discuss any aspect of the study protocol and GCP requirements. Training records were reviewed to ensure investigators and staff were qualified to conduct the study and to document training in GCP. Any staff lacking in GCP training were provided an electronic GCP training module. Documentation of GCP training was confirmed prior to staff participation in the study.

Participants who consented to participate in the study and were administered study treatment, but never subsequently donated a sample for research, were considered “baseline failures”. Data was entered onto the eCRF for all baseline failures.
A list of all concomitant medications for co-morbid conditions taken within 48 hours prior to the TBCB procedure were recorded in the eCRF. The minimum requirement was drug name, date of dosing and, if possible, time of dosing to be recorded.

**SAFETY ASSESSMENTS**

The safety assessments were the monitoring of AEs, clinical laboratory tests, vital signs, ECGs, physical examinations, and post procedure chest X-Ray. Some of these safety assessment procedures e.g., ECG were done as standard of care and the data was not collected for this study.  

The investigator or site staff were responsible for detecting, documenting, and reporting events that met the definition of an AE or SAE. AE information volunteered by the subject, discovered by investigator questioning or detected by other means was collected from the start of study treatment until the follow-up contact. The following information on AEs was obtained:

- Duration (start and stop dates).
- Severity (mild, moderate, severe).
- Causality (reasonable possibility yes/no).
- Actions taken and outcome.

The AE and SAE definitions are provided in the Protocol Appendix 2.

**STATISTICAL CONSIDERATIONS AND ANALYSES**

The planned sample size for this study was 20 participants to enter the study, acknowledging that not all would provide study samples. The sample size was
based on feasibility of recruiting participants in the duration of the study and was considered adequate to achieve the objectives of the study.

Baseline patient characteristics are presented as summary statistics.

Data generated from this study was semi-quantitative MALDI-MS and histology imaging therefore no formal statistical analysis was undertaken, instead only summary statistics has been provided. No formal hypothesis was tested.
6.4 METHODS – PART B: Clinical study samples processing and analysis.

Biopsy Sample Processing

Sample processing supplies (e.g., labels, trays and embedding materials) were provided by GSK. Frozen TBCB and endobronchial forceps biopsy samples taken for research were immediately embedded individually into the Poly[N-(2-hydroxypropyl)-methacrylamide] embedding polymer and frozen as detailed below. For TBCB samples, the specimen was removed from the cryobiopsy probe using warmed gauze and removed from the probe, as quickly as possible to minimise thawing, before embedding.

10 mL of 15% (w/v) Poly[N-(2-hydroxypropyl)-methacrylamide] (pHPMA) was prepared in HPLC grade water and stored at 4°C for at least an hour before use. Prepared pHMA can be stored at 4°C for up to 3 months.

0.7 mL pHMA solution was dispensed to the centre of a disposable mould and the cryobiopsy sample immersed into the solution and immediately placed on dry ice to freeze. Once the solution containing the biopsy sample is frozen, the mould and contents were stored in a suitable, labelled, container/plastic bag in a -80°C freezer.

Sample collection date and ID were completed on the sample label and corresponding sample details were recorded on the Biopsy Sample Processing Worksheet, for each unique sample collected. Samples were shipped on dry ice to GSK in Stevenage for analysis.
**Biopsy Sectioning**

Biopsy sectioning methods were the same for both the pre-clinical and clinical studies.

The frozen block of embedding material containing the biopsy was removed from the mould and mounted onto the chuck of a Leica 3050S Cryostat (Leica Microsystems, Wetzlar, Germany) using carboxymethylcellulose (CMC) (1 % w/v aqueous) ensuring the block was not immersed within the CMC. Consecutive sections of 10 µm thickness were cut and thaw-mounted continuously in the following order: a section mounted onto an indium tin oxide coated glass slide (Bruker Daltonics, Bremen, Germany) for MALDI-MS Imaging and the next section thaw-mounted onto a frosted end microslide (Menzel-Glaser Superfrost®, Thermo Fisher Scientific, Waltham, MA, USA) for Histology, H&E staining. The glass mounted tissue sections were optically scanned in a Super CoolScan 5000ED scanner fitted with a MA-21 slide mount adapter (Nikon Corporation, Tokyo, Japan) to produce a digital image for future reference.

**Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS) analysis**

Following sectioning of biopsy samples for MALDI-MS imaging, the remainder of the biopsy samples were analysed by LC-MS/MS for confirmation of drug presence.
Each biopsy sample was individually thawed, removed from the embedding material and weighed, then homogenised in a fixed volume of water (irrespective of the sample weight) at 5000 rpm in 2 mL Precellys tubes containing ceramic beads (Precellys 24, Berin Instrument). The samples were extracted by protein precipitation and the extracts were analysed by LC-MS/MS (Shimadzu Nexera UPLC, Sciex API6500+; Kinetex EVO 2.6 µm C18 2.1 x 50 mm column).

Ipratropium was identified by LC-MS/MS using the LC retention time of the drug and the predetermined specific mass transitions for ipratropium.

**MALDI MSI analysis**

In the MALDI-MS imaging experiments the ipratropium cation was detected and will be referred to as ipratropium or drug.

Tissue sections were coated with ~1-2 mL of 7 mg/mL CHCA matrix solution in 70 % MeOH/0.2 % TFA (aq.) (Imageprep, Bruker Daltonics). LIFT MS/MS spectra were acquired in positive ionisation mode using a Smartbeam II laser, 200 laser shots per position (RapifleX or UltrafleXtreme MALDI TOF/TOF, Bruker). Mass spectrometer parameters were as per the manufacturers recommended settings, adjusted for optimal performance. Data analysis was carried out using FlexImaging v 4.0.

Predetermined specific mass transitions for ipratropium (m/z 332.2-166.0 and 332.2-123.9) were utilised. Following smoothing and baseline correction, a signal to noise threshold ratio of 3:1 was applied to both fragment ions (166.0 and 123.9) for detection of ipratropium. A spatial resolution of either 30, 100 or 200 µm was utilised and the signal for ipratropium was displayed using a
colour coded ion density map. This detection criterion was based upon the analysis of the control rat lung (Rat #10 - undosed) from the pre-clinical study and provided a sufficient cut-off to eliminate the occurrence of any false positives.

Histopathology

After MALDI-MS imaging analysis was complete, a histopathological assessment of the biopsy section (representing the lung parenchyma) was made, to evaluate the degree of fibrosis and inflammation in the biopsy and therefore the lung. This also allowed the correlation of specific ion images (i.e., drug distribution profiles) with the histological features observed by optical microscopy and digital scanning instruments on the same section. To achieve the histopathological assessment, the MALDI matrix was removed from the sample using ethanol washes and the H&E staining procedure performed, if sample integrity was maintained. At times this can result in poor histology and thus adjacent histology sections were also assessed (having not undergone MALDI-MS).

The biopsy sections were stained with haematoxylin and eosin (H&E) following standard histological procedures derived from Lillie et al (174) The procedure used is outlined below:

1. If a section was not used for MALDI-MS proceed directly to step 2 below. For sections used for MALDI-MS, first wash the section twice using 70% ethanol and then 100% ethanol. This step to removes the matrix that was applied to the slide prior to performing MALDI-MS imaging.
2. If necessary, allow the slides to equilibrate to room temperature.
3. Immerse slides for 1 minute in 10% neutral buffered formalin.
4. Then, 30 seconds in hematoxylin-2
5. Rinse for 1 minute with running tap water
6. Immerse slides for 10 seconds in clarifier solution.
7. Rinse for 1 minute with running tap water
8. Immerse for 1 minute in bluing reagent.
9. Immerse for 15 seconds in Eosin-Y
10. Rinse for 1 minute with running tap water
11. Dehydrate with ethanol.
12. Dehydrate with Xylene
13. Slides should be covered with a coverslip prior to scanning using a suitable glue (e.g., Cytoseal).

Images were captured digitally and scanned at either 20× or 40× magnification (Aperio Scanscope CS, Leica Microsystems, Milton Keynes, UK).
6.5 Results

6.5.1 Clinical Study

Seven participants were enrolled of whom five completed the trial providing six TBCB samples ranging from 4 to 6 mm\(^2\) in size and fourteen endobronchial biopsy samples ranging from 0.75 to 3 mm\(^2\) in size. TBCB samples were taken within 70 mins of the end of ipratropium nebulisation.

Participants’ characteristics are summarized in Table 6.2.
### Ipratropium bromide (N=7)

<table>
<thead>
<tr>
<th>Age in Years [Mean (SD)]</th>
<th>62.1 (5.98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male [n (%)]</td>
<td>3 (43)</td>
</tr>
<tr>
<td>BMI (kg/m²) [Mean (SD)]</td>
<td>31.11 (3.191)</td>
</tr>
<tr>
<td>Height (cm) [Mean (SD)]</td>
<td>168.71 (16.039)</td>
</tr>
<tr>
<td>FVC [Mean (SD)]</td>
<td>2.58 (1.161)</td>
</tr>
<tr>
<td>% predicted FVC [Mean (SD)]</td>
<td>73.75 (12.863)</td>
</tr>
<tr>
<td>FEV1 [Mean (SD)]</td>
<td>2.12 (0.773)</td>
</tr>
<tr>
<td>% predicted FEV1 [Mean (SD)]</td>
<td>77.07 (11.256)</td>
</tr>
</tbody>
</table>

**MDT Diagnosis of study completers:**

<table>
<thead>
<tr>
<th>Study Completers (N=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPF/probable IPF [n (%)]</td>
</tr>
<tr>
<td>NSIP/fibrotic NSIP [n (%)]</td>
</tr>
</tbody>
</table>

**BMI** – Body Mass Index; **FVC** – Forced Vital Capacity; **FEV1** Forced Expiratory Volume at 1 second; **MDT** – Multidisciplinary Team; **IPF**-Idiopathic Pulmonary Fibrosis; **NSIP**- Non-specific Interstitial Pneumonia.

Note 1: ‘One participant had to be re-enrolled.

| Table 6.2: Participant Characteristics |
Two participants were withdrawn at the bronchoscopist’s discretion, prior to research samples being taken, one, due to endobronchial bleeding and the second due to technical difficulties leading to a prolonged procedure.

**Adverse Events**
The adverse events reported for the study are presented in Table 6.3.

**Serious Adverse Events (SAEs)**
Three participants in this study had SAEs reported namely pneumothorax (n=2) and malaise (n=1).

One participant was admitted for observation due to feeling “generally unwell” with headache, sore throat and chest pain after the procedure. The participant remained stable and was discharged two days later. Due to the hospital admission this was classed as a SAE and recorded as malaise.

Two participants developed pneumothorax after the procedure, pneumothorax is a known potential complication of biopsy. Both participants were admitted to hospital for drainage of the pneumothorax.
<table>
<thead>
<tr>
<th>Adverse Events</th>
<th>(N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedural haemorrhage¹</td>
<td>5 (71%)</td>
</tr>
<tr>
<td>Procedural pneumothorax</td>
<td>2 (29%)</td>
</tr>
<tr>
<td>Cough</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>Dry throat</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>Malaise</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>Musculoskeletal chest pain</td>
<td>1 (14%)</td>
</tr>
</tbody>
</table>

¹Bleeding (procedural haemorrhage) is an expected adverse event associated with biopsy procedures. In this study, for one participant the procedure was stopped before biopsies due to bleeding. For all other participants, bleeding was mild and managed as per UCLH routine procedure.

Table 6.3: Summary of Adverse Events
6.5.2 Drug Detection by LC-MS/MS

Ipratropium was detected in all six TBCB samples (Table 3.4). No quantification data is available due to insufficient TBCB or endobronchial control material being available to prepare calibration standards. In addition, an identical liquid volume was used to produce homogenate for each sample irrespective of their differing weights (to aid detection).

Drug was detected by LC-MS/MS in thirteen endobronchial biopsy samples tested.
<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Biopsy Type</th>
<th>Ipratropium Detected (+/-)</th>
<th>Sample Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A</td>
<td>TBCB (Distal)</td>
<td>+</td>
<td>3.4</td>
</tr>
<tr>
<td>4A</td>
<td>TBCB (Distal)</td>
<td>+</td>
<td>20.4</td>
</tr>
<tr>
<td>5A</td>
<td>TBCB (Distal)</td>
<td>+</td>
<td>23.2</td>
</tr>
<tr>
<td>6A</td>
<td>TBCB (Distal)</td>
<td>+</td>
<td>0.1</td>
</tr>
<tr>
<td>6B</td>
<td>TBCB (Distal)</td>
<td>+</td>
<td>7.8</td>
</tr>
<tr>
<td>8A</td>
<td>TBCB (Distal)</td>
<td>+</td>
<td>97.8</td>
</tr>
<tr>
<td>3B</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>3C</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>2.6</td>
</tr>
<tr>
<td>4B</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>4C</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>0.1</td>
</tr>
<tr>
<td>4D</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>1.9</td>
</tr>
<tr>
<td>5B</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>0.1</td>
</tr>
<tr>
<td>5C</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>5D</td>
<td>Endobronchial (Proximal)</td>
<td>No Sample*</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>6C</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>-------------------------</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>6D</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>6E</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>8B</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>8C</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>8D</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
</tr>
</tbody>
</table>

*Endobronchial biopsy sample 5D was lost during sample preparation.

Table 6.4: Summary of ipratropium detection by LC-MS/MS

6.5.3 MALDI-MS Imaging Results

Drug was detected in TBCB samples from four of the five participants using MALDI-MS imaging. Representative figures for the detection of ipratropium in distal and proximal lung section samples from these four participants are shown in Figures 6.2.a – 6.2.d (for TBCB) and Figure 6.3.a-e (endobronchial biopsies). The circled regions in Figures 6.2 represent the drug foci regions meeting the selection criteria for the positive identification and detection of
ipratropium. All regions in Figures 6.3 met the selection criteria for the positive identification and detection of ipratropium.
Figure 6.2a
Figure 6.2b
Figure 6.2c
<table>
<thead>
<tr>
<th>(i) Photo</th>
<th>(ii) Spectra</th>
<th>(iii) Processed MALDI MS Image</th>
<th>(iv) Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.jpg" alt="Photo" /></td>
<td><img src="image2.jpg" alt="Spectra" /></td>
<td><img src="image3.jpg" alt="Processed MALDI MS Image" /></td>
<td><img src="image4.jpg" alt="Histology" /></td>
</tr>
</tbody>
</table>

Figure 6.2d

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Figure 6.2 a-d: Representative MALDI-MS images, histology images and MS/MS for each participant (TBCB samples)

(i) a photograph of the frozen embedded biopsy sample,
(ii) mass spectra showing both fragment ions (at m/z 123.9 and 166.0), obtained at the site of confirmed ipratropium detection (referred to as ipratropium or drug foci).
(iii) the MALDI-MS image (100 µm pixel size) for the biopsy sample section. The signal intensity for the ipratropium fragment ion at m/z 166.0 is represented as a concentration-dependent colour scale – white being highest concentrations.
(iv) The corresponding histology image.

Note: For clarity, the MALDI-MS images for the detection of ipratropium have been adapted and the drug foci regions circled that are above the signal to noise threshold ratio 3:1 for both fragment ions (at m/z 123.9 and 166.0). The approximate location of these foci has been circled on the corresponding histology image.
<table>
<thead>
<tr>
<th>Figure</th>
<th>6.3c</th>
<th>6.3d</th>
</tr>
</thead>
<tbody>
<tr>
<td>(iv) Histology</td>
<td><img src="image1.png" alt="Histology Image" /></td>
<td><img src="image2.png" alt="Histology Image" /></td>
</tr>
<tr>
<td>(iii) MALDI</td>
<td><img src="image3.png" alt="MALDI Image" /></td>
<td><img src="image4.png" alt="MALDI Image" /></td>
</tr>
<tr>
<td>(ii) Spectra</td>
<td><img src="image5.png" alt="Spectra Image" /></td>
<td><img src="image6.png" alt="Spectra Image" /></td>
</tr>
<tr>
<td>(i) Photo</td>
<td><img src="image7.png" alt="Photo Image" /></td>
<td><img src="image8.png" alt="Photo Image" /></td>
</tr>
</tbody>
</table>
Figure 6.3 a-e Representative MALDI-MS images, histology images and MS/MS for each participant (endobronchial samples)

Each representative figure depicts.

(i) a photograph of the frozen embedded biopsy sample,
(ii) mass spectra showing both fragment ions (at m/z 123.9 and 166.0), obtained at the site of confirmed ipratropium detection (referred to as ipratropium or drug foci).
(iii) the MALDI-MS image (100 µm pixel size) for the biopsy sample section. The signal intensity for the ipratropium fragment ion at m/z 166.0 is represented as a concentration-dependent colour scale – white being highest concentrations.
(iv) The corresponding histology image.
TBCB (Distal Lung) MALDI-MS Imaging

Ipratropium was detected in TBCB sections as either a single foci or multiple foci using MALDI-MS imaging (Figure 6.2). The sample shown in Figure 6.2b contains five ipratropium foci. Three of these foci are adjacent to each other and appear to be co-located with an airway.

In another TBCB sample ipratropium loci were observed to localise in the same region in consecutive biopsy sections (Figure 6.4a and 6.4b) suggesting an alignment through the z-plane.
Figure 6.4: Images showing MALDI-MS imaging hit on consecutive sample sections, 4A32 (a) and 4A33 (b).
Co-location of MALDI-MS Imaging and Histology in TBCB

Fibrotic regions were identified in biopsies of four of the five participants as indicated by coalescing areas of poorly cellular eosinophilic fibrillar material (interpreted as collagen). Combining the MALDI-MS images and histology demonstrated co-location of ipratropium with fibrotic regions in the TBCBs of three of the four participants with fibrosis.

Whilst the number of drug foci within the TBCB sections was low, there were examples from three participants, Figures 6.2.a, 6.2.b and 6.2.c where the drug foci were shown to co-locate with areas of fibrosis. This indicates that for these three participants, ipratropium bromide could be deposited in regions of the distal lung where fibrosis was also confirmed.

In TBCB sections from two participants, drug foci were present within abnormal fibrotic areas (Figure 6.5 A-D), possibly co-located with small airway, however, low resolution of the image does not allow a full histological interpretation. One participant (diagnosed with non-specific interstitial pneumonia) did not have abnormal fibrotic areas observed in the research sample, although ipratropium was successfully detected in the TBCB sample from their distal lung.
Figure 6.5 a-d: Co-location of MALDI-MS imaging and Histology in TBCB.

Left: (A) and Zoomed-in region (B) depict the approximate location of the MALDI-MSI hit present within a fibrotic area of TBCB sample 4A31, possibly co-located with a small airway.

Right: (C) and zoomed-in region (D) of TBCB sample 5A23 illustrate lung architecture consistent with pulmonary fibrosis and the approximate location of the MALDI-MSI hits appear to co-locate with a small airway.
MALDI-MS Imaging Results for the endobronchial biopsy (proximal lung)

Endobronchial biopsy samples were taken as a control to confirm drug inhalation by the participant. The levels of ipratropium were expected to be higher in the proximal airways than in the distal lung.

Ipratropium was detected in at least one endobronchial biopsy sample for each of the participants, see Figure 6.3 and Table 6.5. The highest signal intensity and greatest number of drug foci were observed in endobronchial samples Figure 6.3.a and 6.3.c
<table>
<thead>
<tr>
<th>Biopsy Sample ID</th>
<th>Biopsy Type</th>
<th>Number of Sections Analysed</th>
<th>Number of Sections with Drug Observed</th>
<th>% Success</th>
<th>Average % Success for endobronchial samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>4A</td>
<td>Transbronchial</td>
<td>25</td>
<td>5</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4B</td>
<td>Endobronchial</td>
<td>26</td>
<td>5</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>4C</td>
<td>Endobronchial</td>
<td>12</td>
<td>8</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>4D</td>
<td>Endobronchial</td>
<td>8</td>
<td>3</td>
<td>38</td>
<td>34.8</td>
</tr>
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<td>Transbronchial</td>
<td>14</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
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<td>Endobronchial</td>
<td>8</td>
<td>6</td>
<td>75</td>
<td></td>
</tr>
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<td>Endobronchial</td>
<td>8</td>
<td>8</td>
<td>100</td>
<td></td>
</tr>
<tr>
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<td>Endobronchial</td>
<td>4</td>
<td>4</td>
<td>100</td>
<td>90.0</td>
</tr>
<tr>
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<td>Transbronchial</td>
<td>12</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>6B</td>
<td>Transbronchial</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
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<td>Endobronchial</td>
<td>9</td>
<td>1</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>6D</td>
<td>Endobronchial</td>
<td>11</td>
<td>4</td>
<td>36</td>
<td>23.5*</td>
</tr>
<tr>
<td>6E†</td>
<td>Endobronchial</td>
<td>7</td>
<td>0</td>
<td>0†</td>
<td>25†</td>
</tr>
<tr>
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<td>Transbronchial</td>
<td>11</td>
<td>4</td>
<td>36</td>
<td></td>
</tr>
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<td>Endobronchial</td>
<td>7</td>
<td>1</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>8C</td>
<td>Endobronchial</td>
<td>9</td>
<td>2</td>
<td>22</td>
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</tr>
<tr>
<td>8D</td>
<td>Endobronchial</td>
<td>5</td>
<td>3</td>
<td>60</td>
<td>28.6</td>
</tr>
</tbody>
</table>

*Average success rate while incorporating sample 6E is 23.5%.
† Due to issues, we had with generating suitable sections from sample 6E for MALDI-MS imaging we have recalculated the average success for sample 6 excluding the data from biopsy E, updated result is 25%.

Table 6.5 Summary of average drug detection rates per study sample
Comparison of drug detection in samples from distal (transbronchial) and proximal (endobronchial) lung

The non-statistical, non-quantitative comparison reported here was conducted to demonstrate that more drug was detected in the proximal regions of the lung compared to the distal regions. Inhaled drugs emitted from a device generating a distribution of particle sizes are more likely to deposit higher amounts of drug in the proximal lung and larger airways than the distal lung and alveoli (175). The signal for the fragment ions of ipratropium were found to be of greater intensities in endobronchial samples relative to the TBCB samples (Figure 3.3). Table 3.5 summarises the average detection success rate by MALDI-MS imaging per biopsy sample. The drug foci were also greater in number across the endobronchial biopsy samples compared to the TBCB samples. In addition, except for TBCB sample 8A, the proportion of sample sections where drug was detected per biopsy was greater for endobronchial biopsy samples (range 23.5 – 90%) compared to the TBCB samples (range 7 –36%). For sample 8 (diagnosed with NSIP), the average detection frequencies for the TBCB and endobronchial biopsies were similar at 36% and 28.6%, respectively.
6.6 Discussion

This study represents, to the best of my knowledge, the first ever successful detection and localisation of inhaled drug in the distal lung of histologically confirmed fibrotic lung parenchyma in patients with a clinical diagnosis of fibrotic ILD. This was achieved through the combination of the novel, minimally invasive biopsy technique TBCB with the mass-spectrometry techniques, LC-MS/MS and MALDI-MS imaging, as well as histopathology.

A total of seven participants were dosed with ipratropium bromide, with five participants providing both TBCB and endobronchial samples. Two patients did not have any research samples taken due to complications during the procedure (bleeding) for one, and prolonged length of the procedure for the other. LC-MS/MS analysis demonstrated the presence of drug in all participants’ TBCBs, suggesting that ipratropium was able to deposit in the distal lung - the area that is most affected in IPF – in patients with fibrotic lung disease and impaired lung function.

Drug aerosol particle size by medical nebuliser is heterodisperse, therefore containing a mix of different particle sizes. Particle size was not measured as we did not perform any drug delivery quantification. According to product literature the combination of a Porta Neb compressor (Phillips Respironics, Amsterdam, Netherlands) running at 6 L/min with a SideStream aerosolising chamber (Respironics, Tangmere, UK) achieves a mass median diameter of <5 µm in 80% of droplets generated. Salbutamol nebulised using the same compressor/nebuliser configuration gave a mean mass median aerodynamic diameter (MMAD) of 2.2 µm (SD 0.4) and a mean geometric standard deviation
3.45 µm (SD 1.1) (176). Aerosol droplet size influences the location of particle deposition and alveolar deposition peaks at about 1.5 µm (177). It was therefore reasonable to assume that the compressor/nebuliser configuration would create aerosol droplets of sufficiently small size to reach the target tissue.

Using LC-MS/MS requires homogenisation of the tissue hence results in a loss of anatomical and spatial information but allows the analysis of a larger sample and thereby can provide increased sensitivity. Conversely, MALDI-MS imaging provides spatial and regional information but is limited by the small sampling size. Due to the small sampling size used (typically 100 µm x 100 µm) achieving sufficient sensitivity in the clinical study proved difficult and LC-MS/MS analysis was used to confirm drug was present in biopsies. Although current MALDI-MS imaging sensitivity was generally unable to fully profile drug distribution in the TBCBs, it was sufficiently sensitive to detect ipratropium in certain foci. The requirement for the coincident presence of both fragment ions in the MALDI-MS imaging data, at a signal to noise ratio threshold of 3:1 or greater as the threshold for the identification of ipratropium to be positively recorded as well as the fact that the drug in the remaining biopsy fraction was detected by LC-MS/MS, provides increased confidence that ipratropium was detected by MALDI-MS imaging.

MALDI-MS imaging detected ipratropium in four participants’ TBCB samples (Figure 6.2) three of whom also had fibrotic regions identified within the TBCB research samples. All patients had an ultimate diagnosis of a fibrotic ILD (either IPF or fibrotic NSIP) based on MDT discussion considering the clinical,
radiological and histological picture (which obviously included the clinical TBCB taken at time of study procedure).

In some instances, e.g., Figure 6.2.d the foci of ipratropium are not directly overlying the biopsy sample. This is likely due to diffusion/delocalisation of ipratropium from the periphery of the sample section during the sample freezing process within the embedding material and/or during the thaw-mounting process of the sample section onto the glass slide in preparation for MALDI-MS imaging. It is the study teams’ opinion that this still constitutes the positive identification/detection of ipratropium in the sample section.

In all five participants, MALDI-MS imaging detected ipratropium in the endobronchial samples. More ipratropium foci and higher ipratropium signal intensities were detected in the proximal lung samples than distal lung samples even though proximal lung samples were smaller in size. This was expected, as in general inhaled drugs emitted from a device generating a distribution of particle sizes are more likely to deposit higher amounts of drug in the proximal lung and larger airways than the distal lung and alveoli (175). In addition, with only 10-30% of nominal dose expected to reach the lung (due to the efficiency of the nebuliser device) (178) and the estimated surface area of the human lung varying between 50 to 75 m² (179), it is expected to be challenging to detect drug deposited in 5 mm² distal lung TBCB samples and if detected, would likely be close to the limit of the detection of any MALDI-MS imaging technique.

The advent of TBCB has brought translational research opportunities by allowing minimally invasive and rapid access to interstitial lung tissue and therefore the potential to study relatively large distal lung biopsies without the
need for a Video Assisted Thoracoscopic Surgery (VATS) or open surgical approach. A further advantage over surgical acquisition of samples is the fact that participants are self-ventilating throughout the procedure which in this study should lead to a more physiological drug distribution than in ventilated participants. Time from nebulisation to biopsy is also reduced as the participant can be nebulised in the bronchoscopy suite directly before receiving sedation.

LIMITATIONS

This proof-of-concept study has several limitations. There was a difference between the demonstrated detection of ipratropium in the pre-clinical study versus the clinical study, despite using what was considered a scaled dose. The human ipratropium dose of 500 mcg was converted to 0.5 mcg/g in lung tissue by assuming a human lung weight of 1000 g. A similar assumption was made for rat lung weight of 1.5 g and the 0.5 mcg/g lung tissue dose was matched between the species. As this was an experimental study, we were not able to quantify the rat to human “disconnect”; we do not have systemic (plasma) data or quantified human lung concentrations. Indeed, the pre-clinical work was only performed to allow study sample workup and methodologies to be put in place. Possible explanations for the observed “disconnect” could be the effect of impaired lung function of the participants, pulmonary clearance mechanisms, or a degree of wash out of drug due to the administration of topical anaesthetic during the bronchoscopy. In the pre-clinical rat study, the lung levels for ipratropium appeared to be consistent throughout the 5 – 65-
minute period. We assumed that this would be the same in humans, but this may not be the case. The delay of up to 60-70 mins before biopsy may have contributed to some dissolution and absorption of the ipratropium in the airways. However, whilst topically active, ipratropium, as a quaternary ammonium compound, is poorly absorbed (180) but has a reported short systemic half-life of 1.6 hrs.

Exact correlation with the underlying histopathology was sometimes confounded due to delocalization of drug, presumably during sample processing, together with limitations to the histological assessments resulting from the use of the embedding material and section thickness needed for sample preparation. There were several instances where the foci of ipratropium were not directly overlying the biopsy sample, particularly evident in the endobronchial samples. (Figure 6.4). In MALDI-MS imaging experiments it is essential to maintain the original spatial distribution of the drug and thus any diffuse/delocalisation is undesirable. This off-tissue effect is likely caused because the pHPMA is a liquid at 4 °C and the thermal mass of the 0.7mL volume may have been sufficient to thaw the extremities of the TBCBs which at the point of sampling were at between 80 – 89 °C on the cryoprobe. The endobronchial forceps biopsy samples, however, were not frozen at the point of sampling and were smaller in size than the TBCBs and thus would be more prone to thawing, resulting in a greater likelihood of drug diffusion/delocalization occurring during the freezing of the block than observed for the TBCBs.
While this study was able to prove that inhaled ipratropium does deposit in distal, fibrosed lung in participants with ILD, it was not always possible to show the exact location within the biopsy samples with confidence. This proof-of-concept study was operating close to the limits of detection of the current MALDI instrument and therefore was not able to show the potential drug distribution. Therefore, in further studies the use of an increased drug dose and/or greater MALDI-MS sensitivity is recommended.

In this proof-of-concept study, we can present confirmation that inhaled drug therapy is a feasible route of administration for fibrotic ILD. However, further work is needed to encompass the influences of the varying physicochemical properties of different pharmaceutical formulations to be used in IPF to optimise distal delivery. Similarly, development of an inhaled therapy would also require an understanding and evaluation of drug clearance particularly since fibrotic interstitium between the alveolar epithelium and the blood supply would likely impair drug penetration into the blood vessels. Future studies using this unique and the powerful combination of TBCB and Mass Spectrometry have the potential to evaluate the ability of an inhaled, or systemically dosed molecule to reach the lung, and may shorten the early clinical phase of an inhaled drug where target engagement is important to demonstrate early in development.

**Conclusion**
We have demonstrated in this study for the first-time using LC-MS/MS and MALDI-MS imaging that a drug taken via the inhaled route can deposit in distal fibrotic lung tissues. All participants had a fibrotic ILD with overall moderately impaired lung function. To our knowledge, this is the first study to directly assess the deposition of non-radiolabeled drugs to the distal lungs of participants with ILDs and correlating histology with drug deposition in these participants.

Ipratropium was detected in all TBCB, and endobronchial samples tested indicating that drug deposition reached the peripheral lung, a region that is most affected in IPF.

This study, therefore, in addition to the study by Usmani et al, 2018 (175) suggests that ILD participants with established fibrosis can benefit from treatments administered by the inhaled route.

Please see appendix 1 for a copy of the publication generated by this work.
Chapter 7 NEUTROPHIL LYMPHOCYTE RATIO AS A SURVIVAL PREDICTOR IN IPF
7.1 INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a progressive, fatal disorder with a variable disease trajectory. It is at times, especially in the early stages of the disease before characteristic radiological and histological signs are apparent, difficult to distinguish from other fibrosing Interstitial Lung Diseases (ILD). The currently available pharmacological treatments for IPF can only slow the progression of the disease, carry an often unfavourable side effect profile leading to high treatment discontinuation rates and are expensive creating significant economic burden. Prognostification at time of diagnosis is notoriously difficult; some patients gradually deteriorate, some undergo stepwise progression, whilst others decline rapidly. Moreover, much of the prognostic data heralds from an era when the criteria for diagnosing IPF were less well, and differently, defined than at present (181, 182, 183). A prognostic biomarker would guide treatment decisions, timing of lung transplant or end of life care and help patients and clinicians to plan.

Therefore a personal medicine approach of firstly diagnosing patients with IPF accurately and secondly identifying early and/or rapid progressors would alleviate both the physical as well as the economical burden of over-treatment of either mis-diagnosed patients or “slow-progressors”. There is an unmet need for biomarkers to guide a personalised approach to care, as well as for cohort stratification in clinical trials.

One of the most commonly used and validated prognostic scoring systems is the GAP score (Gender, Age, Physiology) (184), however it is a relatively
static model that has been unable to prospectively predict rapidly deteriorating patients, nor is it helpful in assessing treatment response. Only two biomarkers have been validated to refine the GAP staging system by identifying high and low risk patients within a given GAP stage. The first used a 52-gene expression signature, an approach that requires calibration against a control cohort(185), and the second measured glucose uptake in the lung with Positron Emission Tomography (PET) (186). Both biomarkers require specialist expertise and are costly, limiting their practicality. An ideal biomarker would be measurable in the blood using a simple and widely available test and would predict prognosis and potentially response to therapy.

Neutrophil-lymphocyte Ration (NLR) is the ratio of neutrophil count to lymphocyte count. NLR is an ideal biomarker candidate as it is easily calculated from a differential white cell count, is cheap and fast to perform. It is thought to reflect the balance between the innate and adaptive immune systems. A high NLR may suggest a relative increase in neutrophils and a decrease in lymphocytes, which could indicate a shift towards a more pro-inflammatory innate immune response. Conversely, a low NLR may indicate a shift towards a more anti-inflammatory adaptive immune response. Other factors such as medication use, stress, and underlying comorbidities can also influence the NLR with increased levels of cortisol leading to neutrophilia and simultaneous lymphopenia (187). Other hormones and cytokines are also likely involved.
NLR has recently been shown to be an independent prognostic factor in several malignancies and has been shown to indicate severity in other inflammatory processes such as diabetes, cardiovascular and renal disease and COPD (188, 189, 190, 191, 192, 193, 194, 195). A meta-analysis of 22 studies found that elevated NLR was associated with poor overall survival and progression-free survival in patients with solid tumours (196). NLR has also been shown to be associated with cardiovascular disease. A systematic review and meta-analysis of 38 studies found that elevated NLR was associated with an increased risk of cardiovascular events, including myocardial infarction, stroke, and cardiovascular death (193). Moreover, NLR has been shown to predict lung involvement and severity of fibrosis in scleroderma and myositis (197, 198) and was predictive in an IPF study with the composite endpoint of “absolute decline in 6-minute walk test of more than 50 meters or death” at 12 months (199).

Other FBC components have been postulated to play a role in IPF. Platelets are a source of TGFβ1, one of the key drivers of fibrosis, which they can release rapidly on activation (200). The role of platelets and their released mediators has been explored in several fibrotic and tissue remodeling diseases. For example, during pulmonary tuberculosis infection, platelets promote inflammation and ECM degradation (201). Platelets also attenuate liver fibrosis by degrading ECM (202), whilst platelet-derived TGFβ1 is pathogenic in cardiac fibrosis (203). This suggests that the biological roles of platelets and platelet-derived mediators may be tissue or disease specific.
It has been reported that IPF patients have elevated mean blood platelet volume (MPV) compared to healthy controls and MPV may represent a surrogate marker for platelet activation in IPF (204).

It has been shown that platelets and neutrophils act in a synergistic manner to cross the endothelium during inflammation in vitro and in vivo (205). Not only could platelets directly promote fibrosis platelet derived TGFβ1, but they may also stimulate neutrophil recruitment into the lung as a result of lung injury or inflammation. However, it is unclear whether platelets and platelet-derived mediators such as platelet-derived TGFβ1 can impact neutrophil recruitment from the blood into the lung to further potentiate the fibrotic process.
7.1.1 AIMS

This study aims to evaluate the use of the Neutrophil-Lymphocyte Ratio (NLR) as a prognostic biomarker of mortality in IPF.

Primary Objective
To evaluate the optimal NLR ratio to define high and low risk groups in IPF and determine whether NLR can predict time to death/transplant in IPF.

Secondary Objectives
- To determine whether the addition of NLR to an existing risk scoring system (GAP score) improves mortality prediction.
- To determine whether NLR can predict treatment response.
- To determine whether NLR can be modified by different therapy interventions.
- To determine whether other full blood count parameters (e.g., Mean Platelet Value) also independently predict mortality.
7.2 METHODS

STUDY DESIGN

This is a retrospective study to evaluate NLR as an independent mortality risk predictor. The optimal NLR cut-off was determined in a derivation cohort of IPF patients who were prospectively recruited as part of a biomarker study (“Prognostic value of PET/CT/MRI in patients with Fibrotic Disease: Radiological Organ-Specific Function and Quality of Life Correlation” (186)). The initial validation was performed on patient data prospectively collected within the UCLH IPF database. Further external validation was performed using clinical databases from five further UK based tertiary ILD referral centres.

Study Patients

The derivation cohort comprised 71 patients enrolled in an imaging study at the University College London Nuclear Medicine Department investigating the potential of $^{18}$F-FDG –PET/CT to predict mortality in IPF between 2008 and 2018.

The internal validation cohort consisted of 134 patients entered the University College London Hospital Interstitial Lung Disease database between 2006 and 2018. The external validation cohorts consisted of 279 patients entered the Royal Brompton and Harefield NHS Trust (RBH) IPF clinical database between 2006-2018 and a combined validation cohort consisting of 515 IPF patients from The Royal Devon and Exeter (RDE) Hospital ILD Service (300), North Bristol NHS Trust (85), Musgrove Park Hospital – Somerset NHS
Patients were included if they had a diagnosis of IPF confirmed by ILD MDT, baseline pulmonary function tests and Complete Blood Counts available. Patients were excluded if they had concurrent malignancy or a haematological disorder known to affect NLR, had evidence of an infection at the time of FBC (CRP >20, clinical or imaging signs of infection), or were on cytotoxic drugs known to cause bone marrow suppression. Patients in the derivation cohort were also excluded if they were on prednisolone 5mg or more at the time of their FBC.

ELIGIBILITY CRITERIA

Inclusion Criteria

• Patient under the care of the clinical ILD team at UCLH or other participating site with data recorded in the locally held clinical database.
• Participants in any relevant clinical trials with ethics approval for further data analysis

Exclusion Criteria

• Insufficient patient data for analysis available
• Patients with known malignancy or haematological disorders affecting Full blood count.
• Patients with known infection at time of Full blood count
• Patients on cytotoxic drugs known to affect Full blood count.
Outcomes
The primary outcome measure was transplant free survival from FBC till death (all-causes) or transplant in high and low NLR groups. Vital status and patient death were confirmed using patient charts, primary health care physician records and national NHS Spine Authentication Service. The following censoring dates were used as cut-off time point for surviving patients in the respective cohorts: UCLH 28/6/2018, RBH 30/1/2020, RDE combined cohort 12/7/2019.
Secondary outcome was assessment of NLR as an independent mortality predictor independent of GAP index.

DATA JOURNEY

The derivation cohort data journey was as follows: study data already collected from the clinical trial “Prognostic value of PET/CT/MRI in patients with Fibrotic Disease: Radiological Organ-Specific Function and Quality of Life Correlation” is held on their study computers at the Nuclear Medicine department in UCLH in an excel format. A research radiographer checked the data for completeness and accuracy. Fully anonymised data was transferred electronically. Data was cleaned and analysed on a secure, password protected UCL laptop using the statistics program STATA. Anonymised raw data was only shared within the research team at UCL.

The validation cohort data journey was as follows: the clinical research team extracted patient data -including patient demographics (hospital number, date of birth, age, sex), diagnosis, past medical history, drug history, date of diagnosis, pulmonary
function test results, full complete blood count results, inflammatory markers (CRP, ESR), smoking status, treatments, date of death, date of last contact alive with services – from the existing clinical database and clinical records into an excel spreadsheet held on a password protected NHS computer at the access restricted department of Thoracic Medicine, 4th floor Euston Road. The data was fully anonymised by removing date of birth and hospital number and assigning a study ID to each record. This fully anonymised spreadsheet was transferred securely. Non-clinical members of the research team did not have access to the non-anonymised data. Data was cleaned analysed and stored as described for the derivation cohort. Data from additional NHS study sites was transferred securely and fully anonymised to UCL for data analysis and handled as outlined above.

**GAP CALCULATION**

GAP Index was calculated as previously described (184) using the following point system:

- Gender: Male 1, Female 0
- Age (years): ≤ 60 0, 61-65 1, >65 2
- Predicted FVC (Forced vital capacity) ): >75% 0, 50-75% 1, <50% 2.
- Predicted TLCO¹ (Transfer Factor): >55% 0, 36-55% 1, < 35% 2, unable to perform test 3.

¹ The terms TLCO and DLCO are used interchangeably. DLCO stands for Diffusion Capacity of Carbon Monoxide whereas TLCO stands for transfer capacity of the lung for the uptake of carbon monoxide
The summary score gives the GAP Index (ranging from 0-8). Based on the GAP Index each patient falls into one of three GAP stages: Stage 1 (0-3 points), Stage 2 (4-5 points), Stage 3 (6-8 points). GAP stage was not calculated if required data was missing.

**PATIENT CONSENT**

Not applicable to this retrospective data analysis. Consent either already obtained as part of a previous clinical trial performed or waived due to the anonymised anonymisation.

**ETHICAL APPROVALS AND FUNDING**

Ethical approval (and any ensuing amendments) was granted by the HRA and Health and Care Research Wales (HCRW). (REC reference: 18/LO/0937) (appendix 7). Site specific and local R&D approvals were granted by each participating hospital site. The study was sponsored by University College London (UCL).

The study was funded by Breathing Matters and a GSK PhD studentship. The funders had no influence on the study design or conduct.
STATISTICAL ANALYSIS

All analysis was performed using STATA 15 (Stata Corp, College Station, Texas). The analysis population includes patients from separate cohorts providing a feasibility-validation analysis. I, in other words the use of NLR in predicting prognosis was tested in the original cohort and then in an independent (and external) validation cohort to prove that any findings are reproducible and not the result of sampling bias.

Optimal NLR ratio was determined in the derivation cohort in an unbiased fashion by taking the median value and graphically demonstrated by the empirical cumulative distribution function.

Cohorts were analysed individually as well as a pooled cohort.

Summary statistics were used to describe patient characteristics. Fisher’s exact test and unpaired two tailed t-tests were used to calculate significance between different group characteristics. All p-values are reported for two-sided confidence intervals.

SURVIVAL ANALYSIS

Both transplant and death were considered events. A p-value of less than 0.05 was considered statistically significant. All analysis was repeated after removing patients with known prednisolone therapy equal to or greater than 5mg at time of initial FBC. The following survival statistical methods were used to calculate risk of death/prediction of transplant-free survival:
Univariate Cox regression and Kaplan-Meier survival analysis was used to examine the relationship between NLR, NLR category (high/low), GAP stage, GAP Index, age, sex, % FVC predicted, % TLCO predicted, steroid therapy (as a binary variable; present if prednisolone 5mg or more), and transplant-free survival. Significance testing between groups on Kaplan-Meier curves was performed using non-parametric log-rank test. Kaplan-Meier curves for NLR category and GAP (unstratified and stratified for NLR category) were constructed to visually display the results.

Multivariate Cox Regression survival analysis

Multivariate stepwise forward cox proportional hazards regression was used to determine whether NLR (as a continuous parameter) and NLR category (high/low) were independent of the GAP index/stage (and their individual components) and steroids in predicting patient transplant-free survival.

GAP-Plus and GAP Index Plus

The To assess whether the addition of NLR improves accuracy of mortality prediction the combination parameter GAP-Plus was constructed by up-staging patients’ GAP stage by one if they were in the high NLR category (therefore creating a four category GAP stage). GAP Index Plus was GAP Index plus one additional point added for a high NLR reading.
**Prediction accuracy assessment**

To compare predictive performance between parameters and models concordance statistics, as the areas under the receiver operating characteristic (ROC) curve using Harrell’s C-Index were measured. This measures the probability that predicting the outcome is better than chance and allowed head-to-head comparison between the different models.

To closer evaluate NLR predictive performance over time a time-dependent ROC analysis (206) was performed at 6 month intervals to illustrate the change in predictive performance.

**Mortality and platelet count**

IPF patients from the UCLH discovery cohort were assessed for mortality based on blood platelet counts. The diagnosis of IPF was made following clinico-radiological multidisciplinary team review. Survival time was taken from point of first presentation to ILD services to time of death or lung transplant. Patients with platelet counts outside the normal range, known haematological disorders or treatments known to affect platelet count were excluded. Patients were divided into 3 equally sized categories with cut-offs determined bias-free as the lower, middle and upper thirds (tertiles) at 207 and 267 x10⁹/L respectively. This analysis was repeated in the Exeter cohort (performed by local study team).
7.3 Results

Patient characteristics in the individual and pooled cohorts are summarised in Table 7.1 for demographic data available across the whole dataset. Data was not available across the whole data set for ethnicity, smoking status, BMI or other co-morbidities. For the 999 patients in the combined (discovery, validation and additional) cohorts, there were 533 events (death or transplant) recorded.
<table>
<thead>
<tr>
<th></th>
<th>Derivation cohort n=71</th>
<th>Internal validation cohort n=134</th>
<th>External Additional Exeter (all sites) n=515</th>
<th>External Additional RBH n=279</th>
<th>Combined all cohorts. N=999</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age years</strong></td>
<td>71.05 (SD 9.0)</td>
<td>74.7 (SD 8.6)</td>
<td>74.0 (SD 8.6)</td>
<td>69.6 (SD 8.7)</td>
<td>72.7 (SD 8.9)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>62 (87.3%)</td>
<td>107 (79.9%)</td>
<td>380 (73.8%)</td>
<td>219 (78.5%)</td>
<td>768 (76.9%)</td>
</tr>
<tr>
<td>Female</td>
<td>9 (12.7%)</td>
<td>27 (20.2%)</td>
<td>135 (26.2%)</td>
<td>60 (21.5%)</td>
<td>231 (23.1%)</td>
</tr>
<tr>
<td><strong>Lung Function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC (%)</td>
<td>77.8 (17.9) n=70</td>
<td>77.1 (21.1) n=133</td>
<td>82.6 (20.2) n=362</td>
<td>72.9 (16.6) n=278</td>
<td>77.9 (19.5) N= 844</td>
</tr>
<tr>
<td>FEV1(%)</td>
<td>78.0 (16.4) n=69</td>
<td>82.5 (22.3) n=123</td>
<td>86.0 (20.7) n=329</td>
<td>77.2 (16.6) n=266</td>
<td>81.8 (19.6) N=788</td>
</tr>
<tr>
<td><strong>TLco (%)</strong></td>
<td>44.8 (13.9) n=61</td>
<td>47.9 (17.9) n=114</td>
<td>49.9 (15.7) n=274</td>
<td>41.9 (14.2) n=258</td>
<td>46.2 (15.8) N=707</td>
</tr>
<tr>
<td><strong>TLco not done/recorded</strong></td>
<td>8</td>
<td>12</td>
<td>1</td>
<td>21</td>
<td>42</td>
</tr>
<tr>
<td><strong>GAP index mean (SD)</strong></td>
<td>4.2 (1.6) n=69</td>
<td>4.4 (1.5) n=126</td>
<td>3.8 (1.3) n=275</td>
<td>4.3 (1.4) n=279</td>
<td>4.1(1.4) N= 749</td>
</tr>
<tr>
<td><strong>GAP stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20 (29.0%)</td>
<td>34 (27.0%)</td>
<td>124(45.1%)</td>
<td>77 (27.6%)</td>
<td>255 (34.1%)</td>
</tr>
<tr>
<td>2</td>
<td>36 (52.2%)</td>
<td>65 (51.6%)</td>
<td>124 (45.1%)</td>
<td>143 (51.3%)</td>
<td>368 (49.1%)</td>
</tr>
<tr>
<td>3</td>
<td>13 (18.8%)</td>
<td>27 (21.4%)</td>
<td>27 (9.8%)</td>
<td>59 (21.2%)</td>
<td>126 (16.8%)</td>
</tr>
<tr>
<td><strong>NLR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3.9 (3.3)</td>
<td>4.1 (3.6)</td>
<td>3.5(2.8)</td>
<td>4.6 (4.2)</td>
<td>3.9 (3.4)</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>2.9 (2.2-4.1)</td>
<td>3.1 (2.0-4.4)</td>
<td>2.8 (2.1-4.0)</td>
<td>3.2 (2.3-4.8)</td>
<td>2.9 (2.1-4.3)</td>
</tr>
</tbody>
</table>

Table 7.1: Patient characteristics in derivation, validation and additional cohorts.
* Lung Function was taken where available. In some cases, FVC was recorded without FEV1 (the latter is not part of GAP score). **TLco : missing
values were only included in GAPscore if documented as ‘not able to perform’- scoring 3 points. Not done or not recorded meant no GAP index was recorded.
The median NLR in the derivation PET cohort was 2.9 (95% CI, 2.2-4.1) and we used this cut-off to determine high (≥2.9) or low NLR (<2.9). This is graphically demonstrated by the empirical cumulative distribution function (ECDF). (Fig 2.1)

Figure 7.1: NLR cut-off of 2.9 estimated by empirical cumulative distribution function

Median NLR across the additional cohorts was similar with UCLH validation cohort, 3.1 (2.0-4.4), Exeter additional cohorts 2.8 (2.1-4.0), and RBH additional cohort 3.2 (2.3-4.8). The combined cohort of 999 patients had a median NLR of 2.9 (2.1-2.3). When the original NLR cut-off of 2.9 was applied to the combined cohort, increasing age, male sex, and worse lung function parameters were all associated with the high NLR category (Table 7.2).

NLR category and GAP stage or GAP Index association was found to be highly significant (p <0.0001). Lower predicted % TLCO was also significantly
associated with being in the high NLR category, however this data was less complete as gas transfer was not available for all subjects and patients who were unable to perform gas transfer were omitted (in contrast to GAP Index were being “unable” to perform TLCO attracts 3 points).
### Table 7.2: Baseline and clinical characteristics of the patients (combined cohort, n=999) in low (<2.9, n=502) and high (>=2.9, n=497) NLR risk groups

* p values were calculated by unpaired two-tailed t-test except for sex and GAP stage where a Fisher’s exact test was used.
Predicted survival by GAP stage was similar to the observed mortality in the combined patient cohort (Table 7.3).

<table>
<thead>
<tr>
<th>Stage</th>
<th>1-year</th>
<th>2-year</th>
<th>3-year</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.3%</td>
<td>13.2%</td>
<td>24.2%</td>
</tr>
<tr>
<td>(n=255)</td>
<td>(Predicted 5.6%)</td>
<td>(Predicted 10.9%)</td>
<td>(Predicted 16.3%)</td>
</tr>
<tr>
<td>II</td>
<td>13.7%</td>
<td>32.5%</td>
<td>46.3%</td>
</tr>
<tr>
<td>(n=368)</td>
<td>(Predicted 16.2%)</td>
<td>(Predicted 29.9%)</td>
<td>(Predicted 42.1%)</td>
</tr>
<tr>
<td>III</td>
<td>32.4%</td>
<td>64.1%</td>
<td>75.1%</td>
</tr>
<tr>
<td>(n=126)</td>
<td>(Predicted 39.2%)</td>
<td>(Predicted 62.1%)</td>
<td>(Predicted 76.8%)</td>
</tr>
</tbody>
</table>

Table 7.3 Observed (and Predicted) Mortality by GAP Stage for our cohort (n=999) compared to the literature predicted values

For the derivation cohort (n=71) there was a significant difference in the median survival between high NLR (>/=2.9) or low NLR (<2.9) with median survival of 62.1 months (IQR 20.2-na), in the low NLR group (n=36), versus 24.3 months (IQR 11.4-69.8) in high NLR (n=35), p= 0.0125. This increased mortality was confirmed in the validation cohort (n=134) with median survival
in the low NLR group (n=64) of 46.5 months (IQR 16.8-93.50) and in the high NLR group (n=70) of 16.9 months (IQR 9.7-43.4), p= 0.0125.

In the combined cohort of 999, the median survival was 49.8 months (Inter quartile range IQR 24.8-88.3) with an incident rate of 0.017 and a total time at risk of 32288 months. Therefore, a total of 2691 patient years were analysed. Finally, the patients were taken as a and were divided into high NLR (>=2.9) or low NLR (<2.9) at time 0; there was a significant difference in the median survival between high and low NLR groups (p <0.0001). Median survival in the low NLR group (n=502) of 49.8 months (IQR 24.8-88.3), incident rate of 0.013 and a total time at risk of 17707 months; median survival in the high NLR group (n=497) of 35.9 months (IQR 15.1-63.7, incident rate of 0.021 and a total time at risk of 14426 months. (Table 7.4)
<table>
<thead>
<tr>
<th></th>
<th>Derivation cohort UCLH n= 71</th>
<th>Internal validation cohort UCLH n=134</th>
<th>External additional SW&amp;L n= 515</th>
<th>External additional RBH n=279</th>
<th>UCLH validation plus external N=928</th>
<th>Combined Cohort N=999</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low NLR</td>
<td>n=36</td>
<td>n=64</td>
<td>n=297</td>
<td>n=120</td>
<td>n=466</td>
<td>N=502,</td>
</tr>
<tr>
<td>Median survival</td>
<td>62.1</td>
<td>45.3</td>
<td>57</td>
<td>46.5</td>
<td>49.6</td>
<td>49.8</td>
</tr>
<tr>
<td>months (IQR)</td>
<td>(20.2-na)</td>
<td>(16.8-93.5)</td>
<td>(32-157)</td>
<td>(22.6-80.0)</td>
<td>(25.9-89.5)</td>
<td>(24.8-88.3)</td>
</tr>
<tr>
<td>Incident rate</td>
<td>0.013</td>
<td>0.017</td>
<td>0.011</td>
<td>0.017</td>
<td>0.013</td>
<td>0.013</td>
</tr>
<tr>
<td>Total time at risk</td>
<td>1337.0</td>
<td>1630.7</td>
<td>9344</td>
<td>5784.0</td>
<td>16370.7</td>
<td>17707</td>
</tr>
<tr>
<td>(months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High NLR</td>
<td>n=35,</td>
<td>n=70</td>
<td>n=218</td>
<td>n=159</td>
<td>n=462</td>
<td>N=497</td>
</tr>
<tr>
<td>Median survival</td>
<td>24.3</td>
<td>16.9</td>
<td>44</td>
<td>39.8</td>
<td>39.0</td>
<td>35.9</td>
</tr>
<tr>
<td>months</td>
<td>(11.4-69.8)</td>
<td>(19.7-43.4)</td>
<td>(18-121)</td>
<td>(19.8-58.8)</td>
<td>(16.0-63.7)</td>
<td>(15.1-63.7)</td>
</tr>
<tr>
<td>Incident rate</td>
<td>0.026</td>
<td>0.031</td>
<td>0.016</td>
<td>0.023</td>
<td>0.021</td>
<td>0.021</td>
</tr>
<tr>
<td>Total time at risk</td>
<td>975.6</td>
<td>1226.5</td>
<td>5479</td>
<td>6356.6</td>
<td>13450.8</td>
<td>14426</td>
</tr>
<tr>
<td>(months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.0255</td>
<td>0.0125</td>
<td>0.0037</td>
<td>0.0223</td>
<td>&lt; 0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Table 7.4:** Median survival in low (<2.9, n=502) and high (>=2.9, n=497)

NLR risk groups per study site/ cohort

* p values were calculated by two-sided Fisher's exact test
Median survival per GAP stage in the pooled cohort is summarised in Table 7.5. Of note is the wide IQR of the unrecorded GAP cohort which points towards its heterogeneity.

<table>
<thead>
<tr>
<th>GAP Stage</th>
<th>n=748</th>
<th>Incidence rate</th>
<th>Median survival in months (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAP 1</td>
<td>n= 255</td>
<td>0.010</td>
<td>73.7 (36.0-100.9)</td>
</tr>
<tr>
<td>GAP 2</td>
<td>n= 367</td>
<td>0.020</td>
<td>41.0 (20-60.0)</td>
</tr>
<tr>
<td>GAP 3</td>
<td>n= 126</td>
<td>0.037</td>
<td>18.0 (10.0-33)</td>
</tr>
<tr>
<td>GAP unrecorded</td>
<td>n= 250</td>
<td>0.013</td>
<td>60 (22-120.6)</td>
</tr>
</tbody>
</table>

Table 7.5: Median survival per GAP stage

Median survival as stratified by NLR risk category was not significantly different for GAP stage 1 (p =0.245) or 3 (p=0.1381) but was significant for GAP stage 2 (p=0.0127) and for the remaining patients who had no GAP stage recorded due to insufficient lung function data. (p=0.0015; Table 7.6)
<table>
<thead>
<tr>
<th>GAP Stage and NLR category</th>
<th>Incidence rate</th>
<th>Median survival months</th>
<th>Recorded Failures</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAP 1 NLR low n= 154</td>
<td>0.009</td>
<td>76.1 (41.8-100.9)</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>GAP 1 NLR high n= 101</td>
<td>0.011</td>
<td>55.8 (33.2-92.5)</td>
<td>48</td>
<td>.245</td>
</tr>
<tr>
<td>GAP 2 NLR low n= 170</td>
<td>0.017</td>
<td>44 (22.3-65.0)</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>GAP 2 NLR high n= 198</td>
<td>0.023</td>
<td>35.7 (16.6-54.2)</td>
<td>137</td>
<td>0.0127</td>
</tr>
<tr>
<td>GAP 3 NLR low n= 48</td>
<td>0.031</td>
<td>19.5 (13.0-39.4)</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>GAP 3 NLR high n= 78</td>
<td>0.042</td>
<td>16.9 (8.2-33)</td>
<td>67</td>
<td>.1381</td>
</tr>
<tr>
<td>GAP unrecorded NLR low n= 130</td>
<td>0.009</td>
<td>83 (34-120.6)</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>GAP unrecorded NLR high n= 120</td>
<td>0.018</td>
<td>44 (12-74)</td>
<td>51</td>
<td>.0015</td>
</tr>
</tbody>
</table>

Table 7.6: Median survival per GAP stage stratified for low (<2.9) and high (>/=2.9) NLR category, n=999 of which n=250 had unrecorded GAP. *2-sided Fisher’s exact
Similarly, using Kaplan-Meier Survival analysis to stratify patients in the same GAP stage by NLR category (low/high) only showed significant differences in survival between low and high NLR for GAP stage 2 (log rank test, p< 0.0001; Figure 7.2C), but not for GAP stage 1 (log rank test, p=0.1755; Figure 7.2B), or stage 3 (log rank test, p=0.0871; Figure 7.2D).
Figure 7.2: Kaplan-Meier survival curves for all-cause mortality following a diagnosis of IPF with follow up extending to 40 months: A. All patients in combined cohort (n=999) divided into GAP stages 1 (n=255), 2 (n=368) and 3 (n=136); B. Patients in GAP Stage 1 stratified into low (<2.9, n=154) and high (>/=2.9, n=101) NLR category at baseline; C. Patients in GAP Stage 2 stratified into low (<2.9, n=170) and high (>/=2.9, n=198) NLR category at baseline; D. Patients in GAP Stage 3 stratified into low (<2.9, n=48) and high (>/=2.9, n=78) NLR category at baseline. The numbers of patients at risk at 10,20,30,40 months for each of these groups is shown in the table immediately below the survival curves. Differences between survival in patients with different GAP stages 1-3 (Figure 7.2A) reached significance (log rank test, p< 0.0001). Stratifying patients in the same GAP stage by NLR category (low/high) only showed significant differences in survival between low and high NLR for GAP stage 2 (log rank test, p< 0.0001; Figure 7.2C), and not for GAP stage 1 (log rank test, p=0.1755; Figure 7.2B), or stage 3 (log rank test, p=0.0871; Figure 7.2D).
The difference in survival between high and low NLR categories was significant with 232 observed events out of 297.55 expected in the low NLR group versus 301 observed events out of 235.45 expected in the high NLR group (log rank test, p< 0.0001). (Figure 7.3)

Figure 7.3: Kaplan-Meier survival curve for all-cause mortality following a diagnosis of IPF for patients in low (<2.9, n=502) and high (≥2.9, n=497) NLR category at baseline, with follow up of 40 months. The numbers of patients at risk at 10, 20, 30, 40 months for each of these groups is shown in the table immediately below the survival curves. This demonstrates a significant difference in mortality between high and low categories (log rank test, p< 0.0001)
Differences between GAP stages and GAP Index scores reached significance (log rank test, p< 0.0001). The NLR-modified GAP calculation, GAP Index-plus and GAP Stage-Plus (see methods) use a very simple modification of GAP dependent on low (+0) or high (+1) NLR, which was chosen as being memorable and easily used. Survival differences between groups were significant for both GAP Index-plus (HR 1.4, 95% CI 1.29- 1.51, p< 0.0001; figure not shown) with survival differences between the GAP Stage-plus groups of patients also reaching significance (HR 1.80, 95% CI 1.60-1.98; log rank test, p< 0.0001; Figure 7.4).
Figure 7.4: Kaplan-Meier survival curves for GAP Stage-plus categories show all-cause mortality following a diagnosis of IPF with follow up extending to 40 months: A, All patients in combined cohort (n=999) were assigned to GAP Stages-plus (initial GAP Stage plus an additional 1 for patients with high, \( >/\leq 2.9 \), NLR category at baseline): GAP-plus stage 1 (n=154), 2 (n=271) 3 (n=246) and 4 (n=78); The numbers of patients at risk at 10, 20, 30, 40 months for each of these groups is shown in the table immediately below the survival curves. The survival differences between the GAP Stage-plus groups of patients reached significance (HR 1.80, 95% CI 1.60-1.98; log rank test, \( p<0.0001 \)).
**Univariate Cox Regression**

Univariate Cox proportional hazards models of the combined cohort (n=999) showed that patients in the high NLR category group had significantly higher mortality/progression to lung transplant when compared with patients in the low NLR group (HR 1.65, 95% CI 1.39-1.95; p<0.0001; not shown), reflecting their baseline demographics (Table 2). NLR category remained significant when each site’s cohort was considered individually (Figure 7.5). Analysis was repeated in the combined cohort excluding all patients on known steroid therapy with a comparable result (HR 1.50, 95% CI 1.24-1.82; p < 0.0001; data not shown). Univariate regressions for GAP Index, GAP Stage, GAP Index-plus and GAP Stage-plus were all significant (GAP index, HR 1.4, 95% CI 1.3-1.5, p <0.0001; GAP Stage, HR 2.1, 95% CI 1.8-2.4, p <0.0001; GAP Index-plus, HR 1.4, 95% CI 1.29-1.51, p< 0.0001; GAP Stage-plus HR 1.8 , 95% CI 1.6-2.0, p <0.0001. Univariate regression was carried out for all the individual GAP components (age, sex, FVC % pred. TLco % pred.) and was significant for all except sex. Age, HR 1.02, 95% CI 1.1-1.03, p <0.0001; sex, HR 1.2, 95% CI 0.99-1.51, p=0.065; FVC% pred, HR 0.98, 95% CI 0.97-0.99, p <0.0001; TLCO% pred, HR 0.97, 95% CI 0.96 – 0.97, p <0.0001. Cox regression for steroid use was also significant for transplant-free survival, HR 1.71, 95% CI 1.37 - 2.12 p <0.0001. The analysis was then repeated in this cohort but with the exclusion of all those patients who had ever taken oral steroids and showed the same significances.
Figure 7.5: Kaplan Meir survival curves for NLR categories for cohorts shown for each centre show all-cause mortality following a diagnosis of IPF with follow up extending to 40 months. Graphs show patients i: A, derivation cohort, UCLH (n=71); B, validation cohort at UCLH (n=134); C, validation cohort RDE (n=515); D, Validation cohort RBH (n=279) who were assigned to low (<2.9) or high ( 2.9) NLR category at baseline: The numbers of patients at risk at 10, 20, 30, 40 months for each
Multivariate

Multivariate analysis was then performed using these individual components as covariates within the model: age, sex, FVC%, TLco%, GAP Stage, use of steroids, NLR (continuous or binary high/low). This analysis showed that after adjusting for GAP Stage and use of steroids in the combined dataset a high NLR category was independently predictive of mortality/progression to lung transplant (HR 1.36, 95% CI 1.12-1.66; p=0.002). Repeating the analysis using the individual GAP components (age, sex, FVC%, TLco%) as variables and again adjusting for steroids showed similar results (HR 1.26, 95% CI 1.03-1.55; p=0.027). Inputting NLR as a continuous variable was also independently predictive adjusted for the individual GAP components and steroids (HR 1.04, 95% CI 1.01-1.07; p=0.011) as well as when adjusted for GAP stage and steroid use (HR 1.05, 95% CI 1.02 -1.07, p= 0.001). All GAP components, except for sex, continued to be significant when adjusted for each other, NLR and steroid use.

Antifibrotic Therapy

Although, for most of the patients, the baseline FBC predated the use of antifibrotics, some patients were later started on antifibrotics. Patients who had taken antifibrotics for >6 weeks were identified in the Southwest (RD&E, MPH, NBT; n=415) and RBH (n=270) cohorts. In Southwest cohort 275 of 415 (66.3%), and in RBH cohort 231 of 270 (85.6%) patients were recorded to have
taken antifibrotics. Therefore, of a sub-cohort of 685 patients, 606 (73.9%) had taken antifibrotics. Univariate regression for antifibrotic therapy was not significant for mortality (HR 1.01, 95% CI 0.79 - 1.29, p=0.95). Multivariate regression taking into consideration NLR category and antifibrotic therapy showed that antifibrotic use remained a non-significant predictor (HR 1.02, therapy 95% CI 0.79 - 1.30, p= 0.899), whereas NLR category was significant (HR 1.59, 95% CI 1.30 - 1.95, p<0.0001)

PREDICTION ACCURACY – Harrell’s C-INDEX

As expected, the best performing prediction model was based on the component variables making up the GAP, NLR as a continuous variable and adjusted for steroids. Incorporating NLR into GAP staging as GAP Plus and GAP Index Plus increased the model’s ability to predict patient mortality. (See Table 7.7)
<table>
<thead>
<tr>
<th>Cox regression model</th>
<th>C-Index</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UNIVARIATE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAP Stage</td>
<td>0.653</td>
<td>0.628-0.679</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>GAP Index</td>
<td>0.666</td>
<td>0.638-0.694</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NLR Category</td>
<td>0.574</td>
<td>0.550-0.597</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NLR</td>
<td>0.611</td>
<td>0.584-0.638</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>GAP Plus</td>
<td>0.660</td>
<td>0.633-0.687</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>GAP Index Plus</td>
<td>0.672</td>
<td>0.644-0.701</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><strong>MULTIVARIATE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAP Stage</td>
<td>0.671</td>
<td>0.643-0.698</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NLR category</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAP Stage</td>
<td>0.681</td>
<td>0.653-0.709</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NLR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLR, age, sex, FVC% predict, TLCO% predict, steroids</td>
<td>0.713</td>
<td>0.684-0.741</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NLR, age, sex, FVC% predict, TLCO% predict</td>
<td>0.710</td>
<td>0.681-0.738</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NLR category, age, sex, FVC% predict, TLCO% predict, steroids</td>
<td>0.710</td>
<td>0.682-0.738</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NLR category, age, sex, FVC% predict, TLCO% predict</td>
<td>0.707</td>
<td>0.678-0.735</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Table 7.7: Harrell’s C-index performance in the pooled patient cohort including steroid users in various predictive models.
Time-dependent ROC analysis in the pooled cohort for NLR demonstrated the continuous decline of the model’s predictive value with the passing of time. For example, AUC at 6 months is 0.728 which declines to an AUC of 0.598 at 48 months (Figure 7.6)
Change in time-dependent receiver operating characteristic for NLR

Figure 7.6: Ability of baseline NLR to predict all-cause mortality in patients with IPF decreases with time. Time dependent change of AUC and ROC in NLR at 6, 12, 18, 24, 30, 36 and 42 months: AUC, area under the curve; ROC, receiver operator characteristic.
Other full blood count parameters

To assess whether any other FBC parameters previously described to correlate with mortality in IPF we undertook univariate and multivariate cox regression in a pooled discovery cohort of UCLH patients (n=142).

Univariate regression of mean platelet volume was not significant (HR 0.96, 95% CI 0.75 - 1.21, p= 0.76) and adjusting for age, sex, FVC and TLCO in multivariate regression was also not significant (HR 0.96, 95% CI 0.75-1.22, p= 0.73). The same analysis repeated with platelet count as a continuous variable was also not significant (n=145; HR 1.00, 95% CI 0.997-1.003, p= 0.937).

In further analysis I excluded platelet counts outside the normal range (on the assumption that these outliers were due to either measuring errors such as platelet clumping or underlying disease processes such as Idiopathic Thrombocytopenia) and created three equally sized cohorts based on platelet count using an unbiased approach (table 7.8). Mortality was highest in patients with the highest platelet count (>267x10^9/L), and lowest in those with the lowest platelet counts (<207x10^9/L, p = 0.033) (However, this observation using similar methodology was not reproduced in a larger validation cohort of IPF patients from Exeter ILD Services, where there was no significant correlation between blood platelet counts with risk of mortality (p = 0.083). In addition, data from the UK Biobank revealed that patients with lower platelet...
blood counts had a significantly higher risk of mortality \((p < 0.001)\). (unpublished work, C Scotten, University of Exeter). These findings suggest that blood platelet counts do not consistently predict IPF disease progression and are not validated as a prognostic disease biomarker.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Platelet Strata</th>
<th>Mean Age (years ± SD)</th>
<th>Mean FVC* (% predicted ± SD)</th>
<th>% Male</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UCLH</strong></td>
<td>Group 1 (n=71)</td>
<td>73.3 ± 10.3</td>
<td>77.9 ± 21.8</td>
<td>77.5%</td>
</tr>
<tr>
<td></td>
<td>Group 2 (n=72)</td>
<td>73.9 ± 8.4</td>
<td>75.9 ± 18.6</td>
<td>86.1%</td>
</tr>
<tr>
<td></td>
<td>Group 3 (n=71)</td>
<td>72.8 ± 8.3</td>
<td>73.4 ± 19.8</td>
<td>74.7%</td>
</tr>
<tr>
<td><strong>Exeter</strong></td>
<td>Group 1 (n=94)</td>
<td>74.1 ± 8.9</td>
<td>83.0 ± 17.0</td>
<td>89.0%</td>
</tr>
<tr>
<td></td>
<td>Group 2 (n=96)</td>
<td>71.6 ± 8.9</td>
<td>83.0 ± 20.0</td>
<td>81.0%</td>
</tr>
<tr>
<td></td>
<td>Group 3 (n=95)</td>
<td>74.0 ± 8.5</td>
<td>84.0 ± 19.0</td>
<td>57.0%</td>
</tr>
</tbody>
</table>

* FVC Forced Vital Capacity

**Table 7.8** Summary of IPF patients’ characteristics in each platelet strata
7.4 DISCUSSION

IPF is a devastating disease with a variable clinical course. One of the most used prognostic cohort scoring systems is the GAP score. However, the GAP score is difficult to apply to an individual and especially within the moderately severe GAP stage II, outcomes can be very variable between separate patients. There is a drive to identify better tools for individual patient risk stratification. While many individual and composite biomarker candidate scores have been proposed the ideal prognostic marker would be measurable in the serum using a simple and widely available test and would predict prognosis and ideally response to therapy. In this chapter, I have shown that baseline NLR derived from a cheap and widely available routine blood test, identifies two groups of patients with IPF with significant differences in outcome. I further show that NLR can significantly refine the predictive capacity of the clinical GAP index.

In his retrospective study I was able to determine an NLR cut off point to split IPF patients into a high and low risk group for transplant-free survival. I then went on to validate this: first, in an internal UCLH cohort of patients prospectively and consecutively added to our specialist service IPF database; and second in two external IPF cohorts provided by five other ILD specialist centers in England. Furthermore, we were able to show, by applying different statistical models, that NLR is an independent risk factor for mortality and adds precision to an established mortality prediction model, the GAP Index.

Our derivation cohort consisted of prospectively recruited patients to a study
using $^{18}$F-FDG –PET/CT to predict mortality in IPF; this was chosen as it was felt to provide the most reliable data source with fully characterised patients and complete survival data available, as opposed to using traditional models of creating a random derivation cohort out of our existing clinical database.

The median NLR in the derivation cohort was 2.9 which comfortably lies within the expected range of 0.78- 3.53 for a healthy, non-elderly adult found by Forget et al (207) by screening 413 healthy adults, while it is above the mean of 2.24 identified for non-Hispanic white Americans in a large study of 9427 subjects (208). It is known that NLR increases with advancing age as well as in pro-inflammatory states such as smoking, obesity, diabetes and malignancies (190). Indeed, in our combined cohort, raised NLR was significantly associated with increased age, as well as worse lung function, and higher GAP Index. Our collaborators from the University of Exeter have been able to further validate NLR as a predictive biomarker by showing that NLR can predict mortality when applied to IPF patient data held in the UK Biobank (Scotten et al – unpublished).

Moreover, we have been able to show that the addition of NLR data refines the existing GAP score mortality prediction model by using C-index and ROC statistics. As expected, the more granular the data inputted the better the prediction model, hence the increased C-Index for a model using the individual components of the GAP Index rather than an overall score (see Table 7.7).

By using time-dependent ROC analysis (206) we were able to calculate the decline in NLR’s predictive accuracy over time and establish that it is most accurate shortly after it being measured. A similar decline in predictive
accuracy has been shown for GAP and other biomarkers (40, 185).

There was marked heterogeneity between the validation cohorts with the RDE cohort being more recent (2011-2019) with a lower average GAP index, GAP stage and mean and median NLR compared with the other cohorts (see Table 7.1). It is encouraging that NLR mortality prediction was robust across all cohorts despite this heterogenicity.

The difference in median survival stratified by GAP stage was only significant in GAP stage 2 and patients in whom the GAP stage could not be calculated. (See Table 7.6). It was interesting to observe that in the latter the overall median survival of 60 months fell between GAP Stages 1 and 2 (with median survival of 73.7 and 41 months respectively). However, when the patients in this group were stratified according to NLR category a remarkable difference in survival became apparent with median survival of 83 months for the low NLR versus 44 months for the high NLR group. GAP was only not available for patients who had incomplete lung function data, nearly always due to missing TLCO readings. Gas transfer can be impossible to perform for the very sick (hence it is coded as a “3” in the GAP score) but patients and clinicians might also influence whether the test is ordered in the first place due to the knowledge that the patient might struggle to perform the necessary "Manoeuvres" (correct spelling) such as breath holding. Therefore, the absence of TLCO might be a proxy for poor performance status or lung function. It might also represent a data quality issue with the absence of a TLCO value automatically interpreted as “missing” rather than “unable to perform”. This would result in a missing GAP score for the patients. On the flip side the low NLR patient group has a longer median survival than GAP stage
1 indicating that this subgroup was possibly seen as too well to warrant full lung function work-up at the time of presentation.

As I have demonstrated that NLR correlates with lung function one could propose its use as a cheap and quick initial screening test to fast-track high-risk patients for early tertiary care review and urgent lung function. This might be especially relevant in current times of increased pressure and backlog on lung function testing due to the COVID-19 pandemic or in resource-poor settings.

It is unclear why NLR is raised in IPF patients with decreased transplant-free survival. It could be a simple marker for ongoing inflammation. Many ILDs have varying degrees of inflammation and/or fibrosis with one often preceding the other, especially in autoimmune associated ILDs. NLR has been shown to predict development and extent of lung fibrosis, for example in systemic sclerosis, (209) and dermatomyositis/polymyositis (210). Here I demonstrate that NLR is also predictive in IPF, a disease in which inflammation is not thought to play a role, and indeed in which the use of immunosuppression in this disease has been shown to be harmful(10). NLR could be pointing towards a potential role of inflammation in advancing interstitial fibrosis or highlighting a group of patients in which inflammation drives increased mortality from cardiovascular involvement. NLR is prognostic in diseases that involve vascular inflammation (including COVID-19 (211)) which also raises the question of IPF being a vascularly driven disease which has previously been postulated in part due to shared risk factors between cardiovascular disease (male sex, smoking history, diabetes) and IPF. Disordered metabolism of carbohydrates, lipids, proteins, and hormones has been documented in lung,
liver, and kidney fibrosis and metabolic dysregulation has been implicated in the pathogenesis of IPF (212), potentially offering a new target for fibrosis therapy. Concrete evidence that raised NLR leads to more rapid decline in lung function in IPF patients was published by Achaiah et al (213) showing it significantly correlated with an FVC decline >10% per year as well as raised mortality.

Neutrophilic inflammation might directly contribute to the pathogenesis of IPF. It has long been established that increased neutrophils in the BAL of patients with IPF correlates with a poor outcome (214). Molyneaux et al. have shown that BAL neutrophilia is associated with both increased microbiome burden and progressive IPF (185), with subtle changes in the microbiome implicated in the initiation and progression of IPF in the absence of identified infection (185). The increased bacterial burden of IPF appears to be in the airway, proximal to the actual fibrotic remodelling of the parenchyma, with very low levels of bacteria identified in IPF parenchymal lung tissue (215). However, such changes are unlikely to cause increases in systemic neutrophilia and NLR in the absence of overt infection. Though it must be noted that NLR can be elevated without the presence of neutrophilia due to relative lymphopenia. For example, a normal neutrophil range lies roughly (depending on specific laboratory cut-offs) between 2.0 and 7.5 x 10^9/litre, while the range for lymphocytes is 1.0 - 4.0 x 10^9/litre. Hence both neutrophils and lymphocytes values can lie within the normal range and still give an elevated NLR. In this study, I excluded patients diagnosed clinically with infection and started on antibiotics, and those in whom the C reactive protein (CRP) was greater than 20 mg/L.
The lung also plays a crucial role in leukocyte homeostasis. There is increasing support for the theory that the lung may orchestrate the disposal of aged neutrophils, by targeting them for recirculation to, and disposal in, the bone marrow. In a mouse model the inability of the lung to clear aged neutrophils resulted in a pulmonary fibrosis (216). As well as neutrophil activation, other groups have noted phenotypic changes in circulating leukocytes, for example CD28 downregulation on CD4 cells, perhaps reflecting T cell exhaustion, and 4 T cell genes (CD24, ICOS, LCK and ITK) are part of the 52 gene signature that is associated with a poor disease outcome (185).

LIMITATIONS

The main limitations of this retrospective study are linked to missing, and at times, poor quality data. We were lacking basic demographic data such as ethnicity, smoking status, and co-morbidities that were not consistently available across all cohorts. In addition, although all cases were incident IPF and CBC was measured at first appointment of IPF diagnosis, we did not consider time to diagnosis which has been shown to vary considerably in UK (217) Data quality and completeness limitation meant that we could not fulfil secondary endpoints such as change of NLR over time and treatment, and therefore could not correlate high NLR with more rapid decline in lung function.

This issue is illustrated in the above discussion of the missing TLCO data. We were originally planning to evaluate whether NLR predicts response to anti-fibrotic treatment with pirfenidone and nintedanib, as well as whether it can be used as a longitudinal marker of treatment response. This was not
possible due to the lack of consistent longitudinal lung function and FBC data. We were able to demonstrate that antifibrotic use was a nonsignificant predictor of mortality but this needs to be seen in the context of unclear timing of initiation and duration of therapy and the restrictions placed on who can access antifibrotics and at what point in the disease process due to NICE regulations in the UK. We were anticipating this problem and had planned to apply for access to trial data from the large Randomised Controlled Trials (RCTs) of antifibrotics nintedanib and pirfenidone published over the last decade, however it is unclear if the trial data is granular enough in recording lymphocyte count routinely at follow up points and accessing trial data proved to be challenging.

Therefore, NLR should be evaluated as part of a prospective clinical trial (possibly embedded as a secondary endpoint in an IPF or PF-ILD treatment trial).
7.5 CONCLUSION

I have demonstrated and validated that NLR is an independent prognostic biomarker that can be evaluated at diagnosis in patients with IPF. A high NLR (>= 2.9) predicts shorter median survival and refines existing risk scoring systems such as GAP Index. I have demonstrated that blood platelet counts do not consistently predict mortality in IPF patients.

The correlation of NLR with lung function could be used as an early screening tool at the time of IPF referral. This could help set clinical priorities in circumstances where lung function is not easily available, for example due to service strains and referral backlogs, remote location or during the COVID-19 pandemic limiting access to diagnostic tests. It might also be helpful in patients who cannot perform lung function testing.

Going forward, NLR should also be investigated in other fibrotic ILDs as a potential biomarker for a progressive phenotype. Further evaluation of the utility as a prognostic and dynamic longitudinal biomarker is warranted.

Please see appendix 1 for a copy of the publication generated by this work.
Chapter 8 DISCUSSION
Interstitial lung disease (ILD) has an incidence of approximately 57/100,000 per year and is associated with significant morbidity (218). The ILDs consist of a heterogeneous group of diseases with varying amounts of interstitial inflammation and fibrosis (181). There is heterogeneity in outcome, with survival in idiopathic pulmonary fibrosis (IPF) particularly poor. IPF is the most common fibrosing ILD with a median untreated survival of 3 years causing 5000 deaths per year in the UK but rate of progression varies individually. The incidence increases with age and IPF is more common in men. A UK study reported an 11% increase in the annual incidence of IPF between 1991 and 2003 (2). The incidence of IPF will continue to rise in the future due to the aging population. Currently, there is a paucity of management options and patient selection for available treatment must be carefully considered in view of an often unfavourable side effect burden. The timing of treatment initiation and referral for lung transplantation depends in part on making an accurate prognosis.

The work presented in this thesis arises from this environment of uncertainty of diagnosis, rate of progression, treatment response and tolerability. It focuses on less invasive, simpler and economical interventions along the personalised care pathway of an IPF patient. This crucially starts with the correct diagnosis, ideally early in the disease process, aided if required by histology obtained through the comparatively minimally invasive TBCB route. Using TBCB samples the INHALE trial provides proof-of-concept for the delivery of inhaled therapeutic compounds in fibrotic lung disease. Finally, it proposes the use of
NLR as a simple and quick prognostic biomaker in addition to the established GAP score to help guide treatment decisions and follow-up intervals.
8.1 Chapter 1

The introduction ARY Chapter 1 describes the current diagnostic pathway and some of its challenges in the work up of ILD patients. It touches onto the use of both bronchoscopic and surgical techniques to obtain lung biopsies when required. Advances in bronchoscopy mean that we may now be able to get enough information needed by taking lung biopsies through TBCB rather than SLB. Data from this thesis provides a further assessment of the utility of TBCB in the NHS setting and its use not only as a new diagnostic but also as a translational research tool.

The chapter further explores disease prognostication in IPF using available biomarkers and risk models. Accurate disease prognosis at the time of diagnosis and being able to identify rapid progressors is not only valuable from a patient perspective but can inform follow-up intervals, timing of treatment initiation and stratify referral for early lung transplantation if indicated.

In recent years the term progressive-fibrosing ILD (PF-ILD) has been coined to describe fibrosing ILDs other than IPF that continue to progress despite optimal treatment (219). There is growing evidence that PF-ILDs respond to antifibrotic treatment (94, 220). However, as for IPF, we still lack the necessary biomarkers to predict which patients will progress at diagnosis and therefore benefit from early initiation of anti-fibrotic treatment.
8.2 Chapter 2

Chapter 2 reports a systematic literature review of the use of SLB in ILD and therefore sets the scene for the need for alternatives. A systematic literature based on the PRISMA guidelines was performed in 2015 by two independent reviewers. The primary outcomes were mortality and complication rates with secondary outcomes including length of stay, diagnostic yield and treatment changes following SLB. Its results lay out the challenges and shortcomings of the gold standard option for obtaining histology with a considerable mortality and morbidity associated with SLB in ILD. The update of the review in 2020 also demonstrates the issues of single centre case series versus large population-based studies.

While the original review found a mean mortality of 4.9% the range among individual studies was wide with 0- 22.4% mortality reported. At times this led to a skewed narrative of relatively small single centre case series such as Sonobe et al and Bagheri et al (49, 64) reporting low mortality and concluding the safety of SLB in ILD patients. Although the relatively large, and prospective, multi-centre study by Fibla et all with 224 patients did report no fatalities (47). The more recently performed, large cohort-based studies identified mortality rates ranging between 3.9%- 7.1% (84, 85, 86).

The largest and robust study by Hutchinson et al (85) used a United States of America national secondary care dataset to identify 32,022 patients who had a SLBs performed between 2000-2011. They demonstrated a 1.7% in-hospital mortality rate after elective out-patient surgeries, and a 16% death rate for
non-elective cases. The authors estimate that during the timeframe studied about 12,000 SLB for ILD diagnosis were annually undertaken in the United States leading them to conclude that close to 10,000 people had died in the US after SLB during the ten-year study period. As reported in the 2001 paper by Utz et al (67) mortality is further heightened to 16.7% in patients subsequently diagnosed with a UIP pattern on biopsy which often, but not exclusively, correlates with an IPF diagnosis.

While SLB is the histopathological gold standard it does not always provide a histological diagnosis and is prone to sampling error. The systematic review found a mean diagnostic yield of 89% and a range of 70-100%. However, pathological diagnosis was often poorly defined for example classifying none-specific findings of “interstitial fibrosis” or “inflammation” as a diagnostic biopsy sample. This unprecise reporting of histological outcomes is likely due to lack of involvement of a specialist pathologist or face-value acceptance of vague pathology reports without wider ILD MDT discussion. The importance of specialist pulmonary pathologists in the histopathologic interpretation of ILD biopsies was shown in a study by Lettieri et al (221) in which 44 SLB samples were examined by general and specialist pathologists. There was poor concordance between the two groups with different diagnosis in 52.3% of cases (kappa 0.21, P < 0.0001). This led to changes in clinical management in 60.0% of patients highlighting the potential serious clinical consequences of misdiagnosis and subsequently mismanagement without the involvement of specialist expertise in the interpretation of ILD biopsies.

Heterogenicity of pathological findings in Idiopathic Interstitial Pneumonias is well documented and it is hence recommended that surgeons take biopsies
from at least two different lobes. Interpretation of discordant pathology has to be made in view of the whole clinical and radiological picture by a specialised MDT but as a general rule UIP pattern gains “top trump” status in overriding other findings due to research confirming that finding any UIP brings the same grave prognosis as finding UIP exclusively on biopsy (222) (223). This heterogeneity explains that even large, multi-lobe SLBs can miss relevant pathology due to sampling error. This is documented in the literature by Panchabhai et al (224) in a series of 389 explanted lungs that had pre-transplant surgical biopsy data available. In this series 12% of cases had misclassified histology in SLB compared with explanted lung. While these findings may appear to discredit the role of biopsies in the diagnosis of ILDs they should merely act as a reminder that histopathology only forms part of the diagnostic jigsaw compromising of clinical findings, radiology and pathology at specialist ILD MDT discussion – the true diagnostic “gold standard”.

While this thesis has focused on TBCB as the main alternative to standard SLB (VATS or OLB) to attain histology I would also like to briefly highlight novel surgical techniques such as none-ventilating and “awake” VATS. One of the main drivers of mortality following SLB is acute exacerbation of the underlying ILD which is more common in patients with a UIP pattern on histology (i.e., patients more likely to have IPF). This is thought to be in part driven by single lung mechanical ventilation during VATS procedure which causes barotrauma in the ventilated lung. Therefore, new surgical approaches utilising self-ventilating “awake” VATS techniques have recently been reported in several case series (95, 225, 226, 227, 228). Self-ventilated biopsy can be performed either with or without sedation and under local anaesthesia (intercostal block
or epidural). Thorascopic and mini thoracotomy approaches have both been used. The reported studies show low mortality with no 30-day follow up deaths reported and low morbidity, but this must be caveated with the low number of overall procedures reported. Length of hospital stay was overall short, for example Pompeo et al (95) report a mean hospital stay of 1.4 ± 0.7 days in their single centre case series. A multicentre, retrospective outcome analysis published in 2019 covering 112 procedures in 7 centres reported an overall mean length of stay of 2.5 ± 2.7 days with considerable variation between the largest contributing centre (60 patients) and the other six centres (1.3 ± 0.5 days vs 3.9 ± 3.4 days, $P<0.001$). The study reported no mortality, complication rate of 7.1% and conversion to general anaesthesia in 6 cases(229). While the literature for "awake" SLB is so far encouraging further research is required to determine its exact risk benefit profile compared with traditional SLB or alternative methods such as TBCB.

In summary, this systematic review provides a summary of the body of evidence that suggests that standard SLB in ILD carries significant morbidity and mortality, especially for a diagnostic procedure. This clearly demonstrates the need for careful consideration of the need for histology in diagnosis, patient selection and the ongoing search for alternatives to standard VATS biopsies, be those novel surgical techniques such as none-ventilating VATS, TBCB, or advances in imaging and diagnostic biomarkers.
8.3 Chapter 3

While performing the systematic review for SLB I became aware that this was relatively underused in the UK and I also became more aware of the need for careful consideration of whom to biopsy and which technique to use and the general complexities and challenges of ILD diagnosis and the crucial role of the ILD MDT. This led to a snapshot in time benchmarking survey completed in February 2016 exploring the specialist ILD MDT landscape in the UK that is presented in chapter 3. 26 specialist centres responded to the survey.

The arrival of antifibrotic therapy in the form of pirfenidone and nintedanib and the establishment of nationally commissioned ILD services to concentrate the necessary expertise and control prescribing of these costly drugs shifted much ILD work away from secondary care to specialised tertiary services. However, this shift came with challenges in funding specialist ILD MDT activity resulting in considerable strain on specialist services at the time of the survey. Lack of adequate staffing and time led to variation of practice regarding which patients would be discussed and for what purpose (diagnostic versus therapeutic decisions) with more patients being discussed prior to initiation of anti-fibrotic therapy than for immunosuppressive therapy which is likely driven by national guidelines of cost reimbursement.

The survey also tried to establish how often SLB were reviewed during the MDT process. Interestingly, the number of patients referred for SLB from specialist ILD MDTs is far lower than the number of diagnostic SLB performed for benign disease reported by the audit by The Society for Cardiothoracic Surgery in Great Britain and Northern Ireland (98) suggesting that a considerable amount of patients referred for diagnostic SLB have not been
discussed in a specialist MDT therefore potentially leading to unnecessary invasive and at times harmful procedures. This finding, together with the mortality and complication rates reported in chapter 2, makes it imperative that national guidelines mandate that patients are discussed in a specialist ILD MDT prior to surgical referral for consideration of VATS biopsy to avoid unnecessary surgery and ensure adequate follow-up and audit.

The regular composition of the MDTs included a consultant respiratory pathologist in 78% of the responding centres with two centres reporting none specialist pathologists attending the MDT, the potential consequence of poor diagnostic concordance was highlighted by Lettieri et al (221). A thoracic surgeon was only regularly in attendance at 17.4% of MDTs.

All specialist centres felt they did not have enough time to discuss cases which was mainly reported to be due to lack of dedicated administrative support and availability of consultant thoracic radiologists. While most centres were able to discuss all or almost all the referred cases, three services could only discuss the most challenging cases. This finding was concerning in view of the known importance of MDT to make an ILD, especially an IIP, diagnosis. Flaherty et al (34) published their systematic assessment of a step-wise MDT approach nearly 20 years ago. In this seminal study they elegantly demonstrated increasing diagnostic confidence and interobserver agreement with every additional item of information such as HRCT or clinical history. In their study, histology had the largest influence on final diagnosis.
NHS England ILD specialised service specifications set out that specialist centres should “enable integration of clinical services with clinical trials and translational research to ensure on going developments in the care of individuals with these rare diseases” (96). This brief was mostly fulfilled with all but two centres enrolling patients into clinical trials thereby showcasing the power of concentrated expertise in dedicated centres to enable patient access to research trials.

While the results of this survey are by now out of date and would have to be repeated to assess current workload of specialist MDTs it appears anecdotally that ILD services are as strained as ever. This is in part due to the licensing of nintedanib for progressive fibrosing ILD and the removal of the upper FVC limit by NICE in 2023 which has increased the referral pool for antifibrotic therapy. Arguably, the role of histopathology has diminished since the survey was conducted and SLB has fallen further out of favour due to changing diagnostic guidelines and greater awareness of risk.
8.4 Chapter 4

Chapter 4 explored the evidence surrounding TBCB through a review of the existing literature. Unsurprisingly the diagnostic yield of TBCB is lower than SLB but a prospective, multi-centre study (141) demonstrated that TBCB significantly increases MDT diagnostic confidence. The COLDICE study (103), which compared TBCB and SLB directly found histopathological concordance between TBCB and SLB was 70·8% and diagnostic agreement at multidisciplinary discussion 76·9% (0·62, 0·47–0·78). As explored in depth in the systematic review of SLB, the definition of diagnostic yield is inconsistent across studies ranging from pure histopathological diagnosis for specimens reported blinded to all radio-clinical information to complicated, stepwise MDT evaluations of diagnostic confidence with the addition of histopathology.

The published complication and mortality data was encouraging with moderate to severe bleeding reported in 11.5% and pneumothoraces in 10% of 3981 patients captured in this review 30-day mortality was 0.38% which compares favourably to SLB mortality with the obvious caveat of patient selection and reporting biases applying.

Overall, the literature is suggestive that TBCB is an alternative to SLB when histology is required for diagnosis but there are important trade-offs to consider when choosing which technique to use with a balance of safety and diagnostic yield. It is also worth remembering that a non-diagnostic sample can be informative in other ways by excluding certain diagnosis without necessarily
confirming another outright thereby adding to overall confidence of the MDT diagnosis. This was illustrated by Lentz et al. who only achieved a confident histopathology diagnosis in 44.2% of TBCB, but this increased to 68.3% at MDT discussion and nonetheless TBCB changed management in 70.2% (124). Of course, pursuit of tissue might become largely obsolete all together in due course though one can imagine that there will be certain circumstances in which tissue will always be required or desirable.
8.5 Chapter 5

Our own TBCB experience is set out in Chapter 5 in which I detail the setting up of the service at ULCH and the results for 35 patients who underwent biopsy. It is important to point out that all patients had been discussed in our ILD MDT and their accurate diagnosis was deemed to require histology. They overall had significantly impaired lung function with a mean FVC of 81.7% predicted and mean TLCO of 53.4%. A firm pathological diagnosis was established in 23 patients (63.9%); non-specific changes were seen in 8 patients (three non-specific interstitial fibrosis and 5 non-specific inflammation with or without associated fibrosis). Three biopsies were reported as inconclusive. Our pneumothorax rate was high at 27% though still not a complete outlier as several groups have reported rates above 20% such as Kronborg-White (26%) (120), Ramaswamy (20%) (116) or Ravaglia (20%) (89). Pneumothorax rates are increased in patients with a fibrotic ILD and we pre-selected our patients for mostly that phenotype. The one event of severe endobronchial bleeding (2.7%) led to the introduction of the routine use of preventative measures such as an endobronchial blocker and pre-medication with IV tranexamic acid and endobronchial adrenaline.

I discussed the relatively low diagnostic yield and its likely explanation in detail in chapter 5 but the low volume of ILD histology reporting by our histopathologists, their later absence from MDT and unfamiliarity with the new TBCB samples contributed on the histology reporting side; our strict classification of what constitutes a diagnostic sample (for example excluding “interstitial fibrosis”), patient selection limited to challenging cases and overall low volumes of 37 procedures over the course of five years.
Overall, our experience as detailed in the chapter has shown many of the pitfalls of setting up a TBCB for ILD diagnosis in the UK which has a very different culture of lung biopsies and interventional bronchoscopy compared with many European centres. In view of the difficulties setting this up in other UK centres bar The Royal Brompton and the above limitations described to histopathology and procedural expertise I would now advocate for a very small number of dedicated TBCB centres to act as referral centres rather than widespread use of the technique. This would likely provide the best outcomes for patients from a complication rate point of view as well as highest diagnostic yield.
Chapter 6 reports the small, but innovative INHALE study which was the culmination of the work required to establish TBCB at UCLH which is further outlined in chapter 5 and close collaboration with our industry partners GSK and their MALDI-MS imaging expertise. The combination of two novel tools in the form of TBCB and MALDI MS allowed us, for the first time ever, to detect and visually localise inhaled compound in the lung parenchyma of ILD patients with impaired lung function. Histology confirmed colocation of fibrotic areas with inhaled drug in some of these patients and all of them were diagnosed with a fibrotic ILD following MDT discussion. This is an important finding for future drug development in fibrotic lung diseases as it opens the avenue to directly target the diseases parenchyma using inhaled medication and therefore circumnavigating the systemic side effects caused by oral or IV therapy. While drug deposition in IPF patients’ lungs has previously been demonstrated using functional imaging such as in the TOPICAL study (175) which used technetium labelled salbutamol particles this has not been able to give any information on whether drug could penetrate into fibrotic areas on a microscopic level.

Using TBCB over SLB specimens for this work has several advantages such as the minimally invasive approach allowing for a day case procedure, the rapid sample acquisition and instant freezing which allows optimal sample preservation especially for further follow-on work which is currently being undertaken looking at lipid signatures of fibrotic lungs. We are also postulating that the fact that patients are self-ventilating throughout the
procedure should lead to a more physiological drug distribution than in ventilated participants undergoing thoracic surgery which is further complicated by single lung ventilation.

This proof-of-concept study is therefore innovative in several ways – the advent of TBCB as a research tool beyond diagnostics, the use of MALDI-MS to detect inhaled drug in real world patients with significant disease and the resulting implications for ILD drug development and its potential acceleration due to the possibility of taking targeted biopsies for analysis of target engagement.
8.7 Chapter 7

In the study presented in chapter 7 I analysed the NLR in a derivation cohort of patients and identified a median value of NLR that separated this discovery population into a high and low risk group for transplant-free survival with significant differences in mortality. I then investigated the prognostic ability of this NLR cut-off in an internal validation cohort of IPF patients and then in a combined cohort which included the addition of two further IPF cohorts provided by five other ILD specialist service centres in the UK. Furthermore, I showed, using a variety of statistical models, that the NLR is an independent risk factor for mortality, and addition of NLR risk profiles to further refine GAP index cohorts significantly increased the prediction accuracy of this clinical score. Although NLR is, unsurprisingly, even more highly predictive as a continuous measure than as a binary ‘high’ or ‘low’, our aim was to modify the GAP score in a simple memorable way, and so we opted for a simple modification of GAP (+1 for ‘high’; +0 for ‘low’) rather than to create a complex composite score in which absolute NLR is incorporated into GAP. We went on to show that the addition of NLR data to GAP score refines the existing mortality prediction model by using C-index and ROC statistics.

I proved NLR to be a robust predictor of median survival despite the heterogeneity of patients in the contributing external cohorts. It is easy, cheap, and quick as well as adding additional information to the existing risk model GAP. I demonstrated a correlation of NLR with lung function which could be used as an early screening tool at the time of referral to help triage clinic and lung function appointments according to risk especially in this time of
increasing pressures on these services in view of the COVID pandemic. In fact, NLR as a continuous variable was almost as predictive as GAP score (Table 7.7: C-index of 0.66 for GAP Index versus 0.61 for NLR) and easier to generate as there is no reliance on lung function. If faced with limited lung function testing ability it might be prudent to prioritise IPF patients at highest risk based on age, sex and NLR. This might be especially relevant in current times of increased pressure and backlog on lung function testing due to the COVID-19 pandemic and in remote, and resource poor, areas where access to lung function is restricted. In addition, lung function can be influenced by operator, equipment, and patient factors such as sub-optimal manoeuvres whereas FBC analysis maintains objectivity.

There is an extensive body of evidence of the role of neutrophils in IPF. Increased frequency of neutrophils within the BALF of IPF patients has been shown to be an independent indicator of poor prognosis and released mediators such as neutrophil elastase and neutrophil extracellular traps (NETs) can mediate PF pathology (214, 230, 231). The neutrophil-lymphocyte ratio (NLR) is a simple and inexpensive biomarker that reflects the balance between the innate and adaptive immune systems. It has been proposed as a potential biomarker for various diseases, including cancer, cardiovascular disease, and infection.

The search for viable biomarkers has taken advantage of the rapidly expanding knowledge of IPF immunopathogenesis. Aberrant repair processes initiated by repetitive injury to the alveolar epithelium result in an exaggerated tissue remodelling response and fibrosis of the lung parenchyma. Proteins
released from damaged epithelium and collagen degradation products can enter the systemic circulation, acting as markers of disease activity by proxy-the most promising of which include CA-19-9 (232), CA-125 (232) and CCL18 (187). Others that have been investigated include SP-D (233), MMP7 (234), osteopontin (OPN), periostin (PON), ICAM1 (235) and telomere length (236). In addition, neo-epitopes generated by the action of matrix metalloproteinases (MMPs) on collagen can be detected in the serum and Jenkins et al found that 6 of 12 of these were predictive for mortality (237). Other serum biomarkers include CD28, ICOS, LCK, ITK (238) alone or as part of a 52-gene RNA signature (185). More recently, attention has turned to imaging biomarkers including imaging quantification (239), measurements of glucose uptake in the lung with Positron Emission Tomography (PET) (186) and a combination of the two (240).

However, of these, only three biomarkers have been validated to refine the GAP staging system by identifying high and low risk patients within a given GAP stage. The 52-gene expression signature, an approach that requires calibration against a control cohort (185), a composite of OPN, PON, MMP-7 and ICAM-1(235) and the Total-to-Background Ratio (TBR) calculated from 18F-FDG-PET imaging, with only the 52-gene expression validated in multiple independent cohorts.

Previous studies have specifically considered NLR as a biomarker in IPF: Of two small single centre studies; the first (241) found NLR raised in 21 patients with IPF compared to 42 healthy controls but was not prognostic; the second study of 73 patients with IPF and 62 healthy controls found that NLR and monocyte lymphocyte ratio (MLR), but not platelet to lymphocyte ratio (PLR),
associated with IPF and correlated negatively with FVC/ DLco (242). Another study measured NLR in bronchoalveolar lavage (BAL) samples from 59 patients with IPF and found that BAL NLR was inversely correlated with FVC measured at the same time as collection of the BAL sample (243). We presented our discovery and validation cohort of 218 patients in 2018 (244). Our initial findings were taken further by Nathan et al(199), who included 1334 patients involved in ASCEND (Study 016; NCT01366209) and CAPACITY (Studies 004 and 006; NCT00287716 and NCT00287729) as a discovery cohort and placebo-treated IPF patients from two independent Phase III, trials of IFN-γ-1b (GIPF-001 (NCT00047645) and GIPF-007 (NCT00075998) as a validation cohort. Significant trends were observed between baseline NLR and PLR quartiles for various outcomes including physiological decline; respiratory hospitalization; and all-cause mortality. However, the only consistent correlation in the discovery cohort was with baseline NLR and the composite endpoint of ‘absolute decline in 6MWD ≥50 m or death’ at 12 months, a finding that was not tested against the validation cohort. Alongside this other groups were investigating circulating cellular biomarkers in IPF. Significant prognostic effects were found for monocyte count a finding validated in > 7000 patients with IPF from five independent cohorts (245) and >2000 patients from a further four cohorts (246). However, the ability of monocyte count to enhance the predictive accuracy of GAP, although promising has not been validated (247) in clinical cohorts.

The retrospective nature and at times poor data quality, especially when it comes to the correct coding of missing transfer factors, is a significant
limitation. However, the fact that NLR category clearly showed a significant survival difference in the group of patients who did not have GAP scores recorded (usually due to absent TLCO, either as they were unable to perform this or it was not performed which is mostly the case when a patient is deemed to be on the less severe end of the disease spectrum) highlights how robust a prognostic marker it remains even in the absence of full lung function data. Although the use of GAP scoring with the addition of the binary high/low NLR provides an easily applied tool to establish clinical cohorts of patients; GAP/NLR, although an improvement on GAP alone, is still limited when used for individual, rather than cohort, prognostication. The C-index, although improved is still only 0.71 which is lower than other biomarkers used for clinical decision-making. A more robust approach for an individual patient would ultimately necessitate input of more granular data, an approach that underlies scoring systems such as the composite physiological index (CPI)(41)

Future work should include the assessment of NLR in other fibrotic ILDs as a potential biomarker for a progressive phenotype. I originally planned to perform a longitudinal analysis to determine whether NLR changes with anti-fibrotic treatment, but this was unfortunately not possible due to poor data quality. This sort of work would ideally be performed in parallel with an existing clinical trial of a therapeutic agent to ensure data rigidity and cost effectiveness and could evaluate prospectively if NLR changes with therapeutic intervention and whether it could predict disease acceleration and exacerbations.
There is renewed interest in IPF and fILD drug development towards anti-inflammatory and immunomodulating compounds. The recent publication of a phase II trial of a Phosphodiesterase 4 (PDE4) inhibitor in 147 IPF patients found that it prevented FVC decline both as monotherapy and in combination with established antifibrotics at the, admittedly short, 12-week follow-up(248). Therefore, the anti-inflammatory and antifibrotic effects of PDE4 inhibitors may be beneficial in patients with idiopathic pulmonary fibrosis. A phase III trial (ClinicalTrials.gov Identifier: NCT05321069) is currently recruiting with results anticipated in 2025. Given the heterogeneity of the IPF phenotype it is easy to imagine that more “inflammatory” patient cohorts might harness greater benefit from novel anti-inflammatory drugs while more fibrotic phenotypes might be better treated with “pure” antifibrotics. A biomarker like NLR may be able to help treatment decisions in the future.

The global collaborative REMAP-ILD trial (Randomised, embedded, multifactorial, adaptive platform trial for interstitial lung disease) has been set up to investigate the repurposing of existing drugs to treat fibrosing ILD(249). The trial is planning to investigate the immunosuppressives azathioprine and mycophenolate in non-IPF fILD. The hypothesis is that these medications would decrease inflammation and may decrease future fibrosis. While IPF patients will not enroll in this particular trial arm it shows that the need to make the diagnostic distinction between IPF and non-IPF fILD is likely to continue for now and TBCB will continue to play a role in obtaining histology.
8.8 Future Work

When we set out to establish TBCB at UCLH in 2013 it was with the knowledge that a large evidence gap exists. To address this, we developed two trial protocols that were planned to be undertaken as future work:

- LUNG COOL trial: CryOextractiOn of Lung tissue for diagnosis of interstitial LUNG diseases
- LUNG TREATS trial: Diagnosing Interstitial LUNG Disease- Transbronchial cryo lung biopsy versus Video- Assisted Thoracoscopic Surgery

I will briefly set out the trial designs and the challenges of delivering these studies.

LUNG COOL TRIAL DESIGN

Hypothesis
In the diagnosis of Interstitial Lung Disease transbronchial cryo lung biopsy (TBCB) and standard bronchoscopy will reduce the number of VATS lung biopsies and result in healthcare cost savings.

Primary Outcome Measure
Proportion of VATS lung biopsies saved and health care cost savings when using TBCB as first line diagnostic tool compared to VATS biopsy alone.
Secondary Outcome Measures

1) The sensitivity of TBCB for the diagnosis of ILD.
2) The diagnostic accuracy of TBCB for patients with ILD
3) Length of inpatient stay post procedure
4) Complications of TBCB

Summary of Trial Design

This is a single arm trial to evaluate the effectiveness and cost-effectiveness of TBCB and standard bronchoscopy (including bronchoalveolar lavage (BAL) and EBUS TBNA if appropriate) in reducing the number of VATS SLB, which is currently considered the gold standard investigation for obtaining histopathological samples. Comparison of the outcomes (proportion of patients undergoing VATS SLB and associated cost) will be made with patients who have previously all undergone VATS SLB. Since the proportion of patients in the control arm to undergo VATS SLB is already known (100%), a control arm is not required.

See Figure 8.1 for Trial flow chart:
Figure 8.1: COOL LUNG trial design flow chart
Methodology and Trial Intervention

Sample size was calculated as 65 patients assuming an 80% power and two-sided confidence interval of 95% to assess a minimum cost reduction of £770 which was deemed significant. A full economic evaluation protocol would have to be followed to assess costs, including of potential complications of procedures and increased length of stay, for both procedures. This sample size is also sufficient to give the study adequate power to assess whether the proportion of patients undergoing VATS SLB is reduced by 40%, assuming the same power and significance level.

Participants will be newly referred patients over 18 years old, with evidence of ILD on High Resolution Computed Tomography (HRCT) who are deemed to require a SLB for diagnosis. All participants will undergo clinical assessment by an ILD specialist. They will then be discussed at a specialist ILD MDT (Multidisciplinary team meeting). Patients with known causes for their ILD will be excluded. The patients’ HRCT will be reviewed by respiratory radiologists. Patients will be offered trial enrolment if following MDT discussion, no confident clinical diagnosis can be reached, and histopathology is deemed useful, and they have no contra-indications to bronchoscopic TBCB and VATS SLB.

The intervention will involve a bronchoscopy with transbronchial cryoscopic lung biopsy (3-5 samples) of lung parenchyma under fluoroscopy and deep sedation. Standard BAL and EBUS TBNA might also be carried out if clinically indicated.
Histological specimens will then be processed as per usual process in the local department of pathology according to standard protocols with serial sectioning. Pathologists with experience in examining samples of lung parenchyma of ILD patients will report the pathology with knowledge of the clinical scenario, which closely reflects clinical practice. However, they will be blinded to the fact that patient is in a clinical trial, minimising observer bias. Due to the difference in the samples size produced from TBCB and VATS SLB it is not possible to blind the pathologists to the procedure employed.

Further size assessment will be carried out using digital imaging software and the area of alveolar tissue of the specimen will be calculated. If a histopathological diagnosis is reached this will be recorded.

All patients will then be re-discussed in the specialist ILD MDT to ascertain firstly a definitive pathological diagnosis and secondly a consensus MDT diagnosis. There will be a proportion of patients in whom a confident pathological and consensus diagnosis cannot be reached. These patients will then be offered VATS SLB. VATS is performed by specialist thoracic surgeons at the Heat Hospital or Papworth Hospital according to standard clinical practice.

Following VATS SLB patients will again be discussed at MDT; if no consensus diagnosis can be reached at that point patients will be followed up for at least 6 months which time they might undergo further diagnostic tests, start specific treatment or be observed as per their treating clinician’s usual practice. All cases in which no consensus diagnosis could be reached following initial TBCB and VATS SLB will again be reviewed at the end of the follow up period by an MDT panel.
DISCUSSION

The study protocol, and informed consent forms, were reviewed and approved by London Camden and Kings Cross Research Ethics Committee (REC reference: 16/LO/0454) (See appendix 8). In May 2014 the trial was adopted by UCL Clinical Trial Unit who supported an unsuccessful funding application through the Health Technology Assessment Program in October 2014. While exploring alternative funding avenues it became apparent that setting up other UK TBCB centres to allow the sample size to be reached would prove too challenging and no further funding applications were made.

To date we remain one of only two centres in the UK that can perform TBCB. Furthermore, the need for histological diagnosis is diminishing due to changes in diagnostic guidelines, increased reliance on radiology and widened access to antifibrotics removing the need for diagnostic certainty to prescribe therapy.

LUNG TREATS TRIAL DESIGN

Hypothesis

Transbronchial cryobiopsy (TBCB) is equivalent to Video-assisted Thoracoscopic Thoracic Surgery (VATS) lung biopsy in securing a histological diagnosis of Interstitial Lung Disease.

Primary Outcome Measure

Diagnostic accuracy of TBCB compared to VATS lung biopsy.
Secondary Outcome Measures

1) Inter-observer agreement between histopathologists for SLB compared to TBCB.

2) ILD Quality of Life questionnaire assessment and pain scores before and after surgery

3) Inter-observer agreement of diagnosis between clinicians; radiologists; histopathologists before and after surgery

Summary of Trial Design

This is a single arm, observational trial to evaluate the diagnostic accuracy of TBCB compared to conventional VATS surgical lung biopsy. TBCB will be carried out in patients at the time of VATS surgery and taken from the same sites as surgical biopsies.

TBCB and VATS biopsies will then be evaluated by two independent histopathologists. To avoid bias, samples from the same patient using different biopsy techniques will not be reported by the same histopathologist. VATS biopsies will be reported by the local pathologists as is current clinical practice as well as one external pathologist. TBCB will be reported by two independent external pathologists without access to the VATS slides.

See Figure 8.2 for trial flow chart.
Figure 8.2 LUNG TREATS trial design flow chart
Methodology and Trial Intervention

Participants will be newly referred patients over 18 years old, with evidence of ILD on High Resolution Computed Tomography (HRCT) who are deemed to require a VATS lung biopsy for diagnosis. All participants will undergo clinical assessment by an ILD specialist. They will then be discussed at a specialist ILD MDT (Multidisciplinary team meeting). Patients with known causes for their ILD will be excluded. An experienced respiratory radiologist will review each HRCT. The respiratory and radiology consultants participating in the MDT will be asked to record the most likely underlying diagnosis and their confidence in this diagnosis for each patient following MDT discussion. Patients will be offered trial enrolment if following MDT discussion, no confident clinical diagnosis can be reached, and histopathology is deemed useful, and they have no contra-indications to TBCB and VATS lung biopsy.

The intervention will involve a bronchoscopy at the time of VATS with TBCB (3-5 samples) of lung parenchyma under fluoroscopy and deep sedation. All the trial interventions are currently offered as part of standard care to patients at UCLH and UCLH clinical staff will train operators at other study sites as required.

Histological specimens will then be processed as per usual process in the local department of pathology according to standard protocols with serial sectioning. Pathologists with experience in examining samples of lung parenchyma of ILD patients will report the pathology with knowledge of the clinical scenario, which reflects clinical practice. Due to the difference in the samples size produced from TBCB and VATS SLB it is not possible to blind the pathologists to the
procedure employed. The VATS biopsy will be assessed and reported in the centre performing the procedure as well as by one further pathologist in another centre; the TBCB biopsy will be assessed and reported by two independent histopathologists from other centres. If a histopathological diagnosis is reached this will be recorded. Pathologists will also be asked to comment on the quality of the biopsies and record their confidence in the diagnosis.

All patients will then be re-discussed in the specialist ILD MDT to ascertain firstly a definitive pathological diagnosis and secondly a consensus MDT diagnosis. Initially patients will be re-discussed with TBCB pathology results available. Participating respiratory, radiology and pathology consultants will be asked to record the most likely diagnosis in their opinion and their confidence level in this diagnosis before and after MDT discussion once TBCB pathology is available. Then patients will be re-discussed with VATS biopsy pathology available. Participating respiratory, radiology and pathology consultants will again be asked to record the most likely diagnosis in their opinion and their confidence level in this diagnosis. There will be a very small proportion of patients in whom a confident consensus diagnosis cannot be reached. These patients will be followed up for at least 6 months during which time they might undergo further diagnostic tests, start specific treatment, or be observed as per their treating clinician’s usual practice. All cases in which no consensus diagnosis could be reached following initial TBCB and VATS SLB will again be reviewed at the end of the follow up period by an MDT panel.
DISCUSSION

The study protocol was developed after initial assessment by our thoracic surgical colleagues with regards to the volume of diagnostic ILD biopsies performed at UCLH and Bart’s Hospitals. Upon carrying out an audit of the actual numbers performed it became clear that recruitment to fulfill the sample size would be challenging. Furthermore, there was no specific funding in place at the time of our discussions that would have allowed additional operating theatre time for a research procedure. However, the concept of LUNG TREATS and research question asked was very similar to the COLDICE (103) study which recruited prospectively from March 2016 till April 2019 across nine Australian centres and was published in Lancet Respiratory Medicine. It compared diagnostic agreement of TBCB and SLB using a sequential approach under one anaesthetic. Outcomes are discussed in more detail above and in Chapter 4.

I believe the publication of COLDICE confirms that the research question asked by LUNG TREATS was both relevant and important and it is unfortunate that we were unable to run this trial due to lack of established TBCB centres and low volumes of SLB performed for ILD diagnosis in the UK. In contrast to COLDICE, the LUNG TREATS protocol planned further follow-up for cases without a consensus MDT diagnosis which would have added possible resolution to contested cases. The most common conflict of pathology in both COLDICE and Romagnoli et al (104) was one biopsy modality providing histology consistent with IPF in the form of UIP pattern while the other showed features consistent with hypersensitivity pneumonitis. Further follow-up MDT
discussion to review clinical course, radiological development and response to treatment might have been informative.
8.9 Conclusions

The research methods used in this thesis have provided a broad overview of the many issues surrounding clear histological diagnosis as well as prognostication in ILDs. I have provided systematic evidence of the side effects and diagnostic yield of the current gold standard, SLB. I have further explored the current use of SLB as part of the MDT approach to ILD diagnosis in the UK highlighting an important gap in appropriate MDT discussion prior to referral. The setting up of TBCB at UCLH as the first UK centre was underpinned by a literature search, a visiting interventional fellowship and always with the need of further data and imbedded research in mind. The collected data provides an important insight into the potential pitfalls and hurdles to routine use of TBCB in the UK and the need for concentrated specialist services to do so.

The future of both SLB and TBCB is likely one of ever diminishing need for histology in view of trial data confirming that antifibrotic therapy is beneficial in none-IPF PF-ILD, therefore currently negating the need for precise diagnosis in this patient group (94). There might however still be a role for certain specific patient groups, for example in whom a malignancy remains in the differential. Furthermore, it is plausible that new IPF therapy in the form of anti-inflammatory compounds such as PDE4B inhibitors might require
increased characterisation of IPF subtypes into inflammatory or fibrotic. Biomarkers such as NLR could also contribute to patient selection for the targeted and personalised therapy in individual IPF patients. TBCB has also be combined with other bronchosopic techniques such as radial EBUS to assess lung pathologies such as lung nodules (250) or cone-beam navigational bronchoscopy for CT guided real time targeting of biopsy sites to improve precision(251).

The delivery of the INHALE trial in conjunction with GSK utilised this novel technique to further advance potential therapeutic routes in the treatment of fibrotic ILDs. While the trial is small, it is unique, novel and informative and I am proud that we managed to deliver it despite difficulties in recruiting. It also showcases how interventional bronchoscopy can be an important research tool beyond diagnostics and therapeutics.

This thesis also demonstrated that NLR is a quick, robust, reproducible and relevant prognostic biomarker which could form part of the arsenal of initial investigations in the initial work up of IPF patients.

My thesis showcases personalised medicine in ILD management from diagnosis, prognostication to drug delivery. The INHALE and NLR biomarker chapters have been published in peer reviewed journals. The manuscripts form appendix 1.
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APPENDIX 1- PUBLICATIONS ARISING FROM THIS WORK

APPENDIX 2- INHALE PROTOCOL

APPENDIX 3- INHALE CONSENT FORM

APPENDIX 4 - INHALE RESEARCH ETHICS COMMITTEE APPROVAL LETTER

APPENDIX 5 – PROTOCOL SLB SYSTEMATIC LITERATURE REVIEW

APPENDIX 6 – NLR PROTOCOL

APPENDIX 7- NLR HRA RESEARCH ETHICS COMMITTEE APPROVAL LETTER

APPENDIX 8 - LUNG COOL Protocol

APPENDIX 9 – LUNG COOL ETHICS APPROVAL

APPENDIX 10 – PATIENT INFORMATION SHEET TBCB
APPENDIX 1- PUBLICATIONS ARISING FROM THIS WORK
Mass spectrometry detection of inhaled drug in distal fibrotic lung

Theresia A. Mikolasch, Eunice Oballa, Mitra Vahdati-Bolouri, Emily Jarvis, Yi Cui, Anthony Cahn, Rebecca L. Terry, Jagdeep Sahota, Ricky Thakrar, Peter Marshall and Joanna C. Porter

Abstract

Background: Currently the only available therapies for fibrotic Interstitial Lung Disease are administered systemically, often causing significant side effects. Inhaled therapy could avoid these but to date there is no evidence that drug can be effectively delivered to distal, fibroed lung. We set out to combine mass spectrometry and histopathology with rapid sample acquisition using transbronchial cryobiopsy to determine whether an inhaled drug can be delivered to fibrotic, distal lung parenchyma in participants with Interstitial Lung Disease.

Methods: Patients with radiologically and multidisciplinary team confirmed fibrotic Interstitial Lung Disease were eligible for this study. Transbronchial cryobiopsies and endobronchial biopsies were taken from five participants, with Interstitial Lung Disease, within 70 min of administration of a single dose of nebulised ipratropium bromide. Thin tissue cryosections were analysed by Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry imaging and correlated with histopathology. The remainder of the cryobiopsies were homogenised and analysed by Liquid Chromatography—tandem Mass Spectrometry.

Results: Drug was detected in proximal and distal lung samples from all participants. Fibrotic regions were identified in research samples of four of the five participants. Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry imaging showed co-location of ipratropium with fibrotic regions in samples from three participants.

Conclusions: In this proof of concept study, using mass spectrometry, we demonstrate for the first-time that an inhaled drug can deposit in distal fibrotic lung parenchyma in patients with Interstitial Lung Disease. This suggests that drugs to treat pulmonary fibrosis could potentially be administered by the inhaled route.

Trial registration: A prospective clinical study approved by London Camden and Kings Cross Research Ethics Committee and registered on clinicaltrials.gov (NCT03136120)

Keywords: MALDI-MS imaging, Transbronchial cryobiopsy, Drug distribution, Interstitial fibrosis

Background

The interstitial lung diseases (ILDs) are a group of over 200 lung disorders that are characterised by interstitial fibrosis, and lead to declining lung function, respiratory failure and ultimately death. The most severe fibrotic (f) ILD is Idiopathic Pulmonary Fibrosis (IPF).

Two oral drugs, pirfenidone and nintedanib, are licensed for the treatment of IPF and have now been shown to have benefits in other fILDs [1] but both have limiting adverse effects. Inhaled therapy for ILD offers the advantage of drug delivery direct to the lung, thereby minimising systemic exposure and associated side effects. However, lung deposition, absorption and local therapeutic response may be altered in the fibrotic lung [2].
Assessment of lung drug levels using bronchoscopic lavage has become a critical component of inhaled drug development but lacks spatial information of the site or region of deposition. The advent of transbronchial cryobiopsy (TBC) to sample the lung parenchyma for diagnosis of ILD allows important histological information of inhaled drug distribution using a minimally invasive bronchoscopic technique [3]. TBC potentially also allows more rapid lung tissue sampling following drug inhalation, compared to traditional surgical lung biopsies therefore shortening the time during which the inhaled drug can be cleared from the lung before analysis. Furthermore, participants are not subject to mechanical ventilation which could theoretically alter inhaled drug distribution in surgical participants.

Liquid Chromatography—tandem Mass Spectrometry (LC–MS/MS) is traditionally used for the analysis of homogenised tissue samples and therefore any spatial information regarding drug distribution within the tissue is lost. In contrast, Matrix Assisted Laser Desorption Ionisation—Mass Spectrometry (MALDI-MS) imaging allows detection and characterisation of molecules from tissue [4–15] and supports the spatial visualisation of drug distribution in tissue samples. Analysing the same TBC biopsy with a combination of LC–MS/MS, MALDI-MS imaging and histopathology can therefore allow minimally invasive assessment of drug distribution within the diseased fibrotic lung.

Inhaled ipratropium was chosen for this study for several reasons: it is a quaternary ammonium compound and therefore strongly positively charged which facilitates detection with MALDI-MS; during the feasibility stage, different drugs (anti-cholinergics, beta-agonists, steroids, and mast cell stabilisers) were tested for of detection using MALDI and secondary-ion mass spectrometry (SIMS) and only ipratropium bromide had good sensitivity for both; ipratropium has been used extensively with robust safety data; and the high therapeutic index and wide therapeutic window of ipratropium also afforded us the ability to increase drug dosing if required. Fehniger et al. [15] have previously demonstrated inhaled ipratropium distribution using MALDI-MS imaging in the proximal airways of patients with suspected airway obstruction or tumours. Ipratropium readily ionises and the MS/MS fragmentation pattern produces two major fragment ions (at m/z 166.0 and 123.9, (Additional file 1: Fig. E1).

In this clinical study, having carried out a single pre-clinical enabling study, we combined, for the first time, rapid distal sample acquisition using TBC with the mass spectrometry modalities of LC–MS/MS and MALDI-MS imaging, together with histopathology to demonstrate inhaled drug delivery to fibrotic, distal human lung parenchyma in participants with diagnosed ILDs. Whilst this study was designed as a proof of concept, with a low participant number (n=5), we are able to present confirmation that inhaled drug therapy is a feasible route of administration for fibrotic ILD, which could avoid the significant systemic side effects of current oral therapy. To our knowledge this is also the first time that TBC has been used in translational research.

Methods
Pre-clinical support study in wistar han rats
All animal studies were ethically reviewed and carried out in accordance with UK Animals (Scientific Procedures) Act 1986, European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

A scaled dose of ipratropium bromide, equivalent to the clinical dose, was nebulised to male Wistar rats for 5 min. Terminal lung samples were taken at varying time points up to 65 min post-dose and 5 mm ex-vivo biopsies were embedded into material suitable for MALDI-MS imaging.

For further details of the pre-clinical animal work please see Additional file 1.

Clinical study
We conducted a prospective clinical study approved by London Camden and Kings Cross Research Ethics Committee and registered on clinicaltrials.gov (NCT03136120) at University College London Hospital (UCLH), London, United Kingdom and sponsored by GlaxoSmithKline. Participants over the age of 18 with suspected ILD and requiring TBC for further diagnostic assessment, as determined by the ILD multidisciplinary team, were eligible to participate (see Additional file 1 for full exclusion/inclusion criteria). Most patients had moderately impaired lung function (FVC between 50 and 80 percent of predicted). Patients with severe ILD were excluded due to risks of additional biopsies in this patient group. Seven participants were enrolled between November 2017 and November 2018.

All participants received a single dose of 500 mcg nebulised ipratropium bromide (Ivax Pharmaceuticals, London, UK) 1 h prior to bronchoscopy. TBCs for diagnosis were taken ahead of the additional (one or two) TBC research samples. Up to three endobronchial forceps biopsy samples were also taken as positive controls to confirm drug inhalation by the participant (see Additional file 1 for full procedural and biopsy collection details).
Liquid chromatography—tandem mass spectrometry (LC–MS/MS) analysis
Following sectioning of biopsy samples for MALDI-MS imaging, the remainder of the biopsy samples were analyzed by LC–MS/MS for confirmation of drug presence. For more details see Additional file 1.

MALDI MSI analysis
For experimental conditions and more details see Additional file 1.

In the MALDI-MS imaging experiments the ipratropium cation was detected and will be referred to as ipratropium or drug. Predetermined specific mass transitions for ipratropium (m/z 332.2–166.0 and 332.2–123.9) were utilised. Following smoothing and baseline correction, a signal to noise threshold ratio of 3:1 was applied to both fragment ions (166.0 and 123.9) for detection of ipratropium. A spatial resolution of either 30, 100 or 200 µm was utilised and the signal for ipratropium was displayed using a colour coded ion density map.

Histopathology
Biopsy sections were stained with haematoxylin and eosin (H&E) following standard histological procedures [16]. Images were captured digitally and scanned at either 20 × or 40 × magnification (Aperio Scanscope CS, Leica Microsystems, Milton Keynes, UK).

Results
Pre-clinical study in rat
In our pre-clinical study, we demonstrated good MALDI-MS sensitivity for ipratropium with widespread distribution of the drug in the lung using both 30 µm and 200 µm spatial resolution at all timepoints (Fig. 1).

Ipratropium was also measured in rat lung sample sections by LC–MS/MS with the mean amount across samples from all timepoints, up to 65 min post administration, in the low pg/section region. This pre-clinical study demonstrated that the sample handling methods used and the MALDI-MS imaging detection limits for ipratropium in lung tissue were suitable.

Fig. 1 Pre-Clinical Study in rats—MALDI-MS imaging of a 16 µm thick tissue section of rat lung and a 5 mm punched biopsy of rat lung, taken 65 min after a nebulised administration of ipratropium. Top Left—Photo of the region of the tissue from where the section had been cut (after removal of multiple punched biopsies). Top Right—MALDI-MS Image showing the distribution of the m/z 166 fragment ion, representative of ipratropium in Rat 7, section 13 (200 µm spatial resolution). The signal intensity for the ipratropium fragment ion at m/z 166.0 is represented as a concentration-dependent colour scale—white being highest concentrations. Bottom Left to Right: a Photo of 5 mm punched biopsy from rat lung, b Optical Image (digitally scanned image of rat lung section), c MALDI-MS image, 200 µm spatial resolution (and Signal Intensity Scale bar), d Histology image (Consecutive section)
Clinical study
Seven participants were enrolled of whom five completed the trial providing six TBC samples ranging from 4 to 6 mm$^2$ in size and fourteen endobronchial biopsy samples ranging from 0.75 to 3 mm$^2$ in size. TBC samples were taken within 70 min of the end of ipratropium nebulisation.

Participants’ characteristics are summarized in Table 1. Representative CT scans are shown at site of biopsies (Right Lower Lobe in all cases) from 4 of the patients in Fig. 2.

Two participants were withdrawn at the bronchoscopist’s discretion, prior to research samples being taken, one, due to endobronchial bleeding and the second due to technical difficulties leading to a prolonged procedure.

Adverse events
The adverse events (AE) reported for the study are presented in Table 2.

Three participants in this study had serious AEs reported namely pneumothorax (n = 2) and malaise (n = 1).

Drug detection by LC–MS/MS
LC–MS/MS was carried out. Ipratropium was detected in all six TBC samples tested (Table 3). No quantification data is available due to insufficient TBC or endobronchial control material being available to prepare calibration standards. In addition, an identical liquid volume was used to produce homogenate for each sample irrespective of their differing weights (to aid detection).

Drug was detected by LC–MS/MS in thirteen endobronchial biopsy samples tested.

MALDI-MS imaging results
Drug was detected in TBC samples from four of the five participants using MALDI-MS imaging. Representative figures for the detection of ipratropium in distal and proximal lung section samples from 4 participants are shown in Figs. 3 and 4 respectively. The circled regions in Fig. 3 (and all regions in Fig. 4) represent the drug foci regions meeting the selection criteria (see Additional file 1) for the positive identification and detection of ipratropium.

TBC (distal lung) MALDI-MS imaging
Ipratropium was detected in TBC sections as either a single foci or multiple foci using MALDI-MS imaging (Fig. 3). The sample shown in Fig. 3B (iii) contains five ipratropium foci. Three of these foci are adjacent to each other and appear to be co-located with an airway.

Ipratropium loci were observed to localise in the same region in consecutive biopsy sections (Fig. 5i and ii) suggesting an alignment through the z-plane.

Co-location of MALDI-MS imaging and histology in TBC
Fibrotic regions were identified in biopsies of four of the five participants as indicated by coalescing areas of

Table 1  Summary of participants’ characteristics

<table>
<thead>
<tr>
<th></th>
<th>Ipratropium bromide (N* = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in Years [Mean (SD)]</td>
<td>62.1 (5.98)</td>
</tr>
<tr>
<td>Male [n (%)]</td>
<td>3 (43)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$) [Mean (SD)]</td>
<td>31.11 (3.191)</td>
</tr>
<tr>
<td>Height (cm) [Mean (SD)]</td>
<td>168.71 (16.039)</td>
</tr>
<tr>
<td>FVC [Mean (SD)]</td>
<td>2.58 (1.161)</td>
</tr>
<tr>
<td>% predicted FVC [Mean (SD)]</td>
<td>73.75 (12.863)</td>
</tr>
<tr>
<td>FEV1 [Mean (SD)]</td>
<td>2.12 (0.773)</td>
</tr>
<tr>
<td>% predicted FEV1 [Mean (SD)]</td>
<td>77.07 (11.256)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MDT diagnosis of study completers</th>
<th>Study completers (N = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPF/probable IPF [n (%)]</td>
<td>3 (60)</td>
</tr>
<tr>
<td>NSIP/fibrotic NSIP [n (%)]</td>
<td>2 (40)</td>
</tr>
</tbody>
</table>

BMI body mass index, FVC forced vital capacity, FEV1 forced expiratory volume at 1 s, MDT multidisciplinary team, IPF idiopathic pulmonary fibrosis, NSIP non-specific interstitial pneumonia

Note 1: One participant had to be re-enrolled

Note 2: As this was a non-quantitative, proof of concept study the impact of the participant’s characteristics was not designed to be taken into consideration.
poorly cellular eosinophilic fibrillar material (interpreted as collagen). Combining the MALDI-MS images and histology demonstrated co-location of ipratropium with fibrotic regions in the TBCs of three of the four participants with fibrosis.

Whilst the number of drug foci within the TBC sections was low, there were examples from three participants, Figs. 3A (iv), B (iv) and C (iv) where the drug

Table 2  Summary of adverse events

<table>
<thead>
<tr>
<th>Preferred term</th>
<th>Ipratropium bromide (N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any event, n (%)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>Procedural haemorrhage(^1)</td>
<td>5 (71)</td>
</tr>
<tr>
<td>Procedural pneumothorax</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Cough</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Dry throat</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Constipation</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Malaise</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Musculoskeletal chest pain</td>
<td>1 (14)</td>
</tr>
</tbody>
</table>

\(^1\) Bleeding (procedural haemorrhage) is an expected adverse event associated with biopsy procedures. In this study, for one participant the procedure was stopped before biopsies due to bleeding. For all other participants, bleeding was mild and managed as per UCLH routine procedure.

Table 3  Summary of ipratropium detection by LC–MS/MS

<table>
<thead>
<tr>
<th>Biopsy sample ID</th>
<th>Biopsy type</th>
<th>Ipratropium detected (±)</th>
<th>Sample weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A</td>
<td>TBC (Distal)</td>
<td>+</td>
<td>3.4</td>
</tr>
<tr>
<td>4A</td>
<td>TBC (Distal)</td>
<td>+</td>
<td>20.4</td>
</tr>
<tr>
<td>5A</td>
<td>TBC (Distal)</td>
<td>+</td>
<td>23.2</td>
</tr>
<tr>
<td>6A</td>
<td>TBC (Distal)</td>
<td>+</td>
<td>0.1</td>
</tr>
<tr>
<td>6B</td>
<td>TBC (Distal)</td>
<td>+</td>
<td>7.8</td>
</tr>
<tr>
<td>8A</td>
<td>TBC (Distal)</td>
<td>+</td>
<td>97.8</td>
</tr>
<tr>
<td>3B</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>3C</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>2.6</td>
</tr>
<tr>
<td>4B</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>4C</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>0.1</td>
</tr>
<tr>
<td>4D</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>1.9</td>
</tr>
<tr>
<td>5B</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>0.1</td>
</tr>
<tr>
<td>5C</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>5D</td>
<td>Endobronchial (Proximal)</td>
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</tr>
<tr>
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<td>+</td>
<td>&lt;0.1</td>
</tr>
<tr>
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<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>6E</td>
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<tr>
<td>8B</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>8C</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>&lt;0.1</td>
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<tr>
<td>8D</td>
<td>Endobronchial (Proximal)</td>
<td>+</td>
<td>0.5</td>
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</table>

\(^*\)Endobronchial biopsy sample 5D was lost during sample preparation.

Fig. 2  Representative CT scans from 4 patients prior to cryobiopsy from Right Lower Lobe in each case: A Patient 001. B Patient 003. C Patient 004. D Patient 006.
Fig. 3  Representative MALDI-MS images, histology images and MS/MS for each participant. (TBC Samples). Each representative figure depicts the MALDI-MS image (100 µm pixel size) for the biopsy sample section and its corresponding histology image, a photograph of the frozen embedded biopsy sample and mass spectra showing both fragment ions (at m/z 123.9 and 166.0), obtained at the site of confirmed ipratropium detection (referred to as ipratropium or drug foci). For clarity, the MALDI-MS images for the detection of ipratropium have been adapted and the drug foci regions circled that are above the signal to noise threshold ratio 3:1 for both fragment ions (at m/z 123.9 and 166.0). The approximate location of these foci has been circled on the corresponding histology image.
foci were shown to co-locate with areas of fibrosis. This indicates that for these three participants, ipratropium bromide could be deposited in regions of the distal lung where fibrosis was also confirmed.

In TBC sections from two participants, drug foci were present within abnormal fibrotic areas (Fig. 5A–D), possibly co-located with small airway, however, low

(See figure on next page.)

**Fig. 4** Representative MALDI-MS images, histology images and MS/MS for each participant (endobronchial samples). Each representative figure depicts the MALDI-MS image (100 µm pixel size) for the biopsy sample section and its corresponding histology image, a photograph of the frozen embedded biopsy sample and mass spectra showing both fragment ions (at m/z 123.9 and 166.0), obtained at the site of confirmed ipratropium detection (referred to as ipratropium or drug foci)

**Fig. 5** Images showing MALDI-MS imaging hit on consecutive sample sections, 4A32 (i) and 4A33 (ii) and approximate location of MALDI hit (middle). Bottom: A Co-location of MALDI-MS imaging and Histology in TBC and Zoomed-in region B depict the approximate location of the MALDI-MSI hit present within a fibrotic area of TBC sample 4A31, possibly co-located with a small airway C and zoomed-in region D of TBC sample 5A23 illustrate lung architecture consistent with pulmonary fibrosis and the approximate location of the MALDI-MSI hits appear to co-locate with a small airway
Fig. 5 (See legend on previous page.)
resolution of the image does not allow a full histological interpretation.

One participant (diagnosed with non-specific interstitial pneumonia) did not have abnormal fibrotic areas observed in the research sample, although ipratropium was successfully detected in the TBC sample from their distal lung.

MALDI-MS imaging results for the endobronchial biopsy (proximal lung)

Endobronchial biopsy samples were taken as a control to confirm drug inhalation by the participant. The levels of ipratropium were expected to be higher in the proximal airways than in the distal lung.

Ipratropium was detected in at least one endobronchial biopsy sample for each of the participants, see Fig. 4 and Table 4. The highest signal intensity and greatest number of drug foci were observed in endobronchial samples Fig. 4A (iii) and C (iii).

Comparison of drug detection in samples from distal (transbronchial) and proximal (endobronchial) lung

The non-statistical, non-quantitative comparison reported here was conducted to demonstrate that more drug was detected in the proximal regions of the lung compared to the distal regions. Inhaled drugs emitted from a device generating polydisperse particle sizes are more likely to deposit higher amounts of drug in the proximal lung and larger airways than the distal lung and alveoli [17].

The signal for the fragment ions of ipratropium were found to be of greater intensities in endobronchial samples relative to the TBC samples (Fig. 4). Table 4 summarises the average detection success rate by MALDI-MS imaging per biopsy sample. The drug foci were also greater in number across the endobronchial biopsy samples compared to the TBC samples. In addition, except for TBC sample 8A, the proportion of sample sections where drug was detected per biopsy was greater for endobronchial biopsy samples (range 23.5–90%) compared to the TBC samples (range 7–36%). For sample 8 (diagnosed with NSIP), the average detection frequencies for the TBC and endobronchial biopsies were similar at 36% and 28.6%, respectively.

Discussion

Here we report the first ever successful detection and localisation of inhaled drug in the distal lung of histologically confirmed fibrotic lung parenchyma in participants with a clinical diagnosis of fibrotic ILD. This was achieved through the combination of TBC, LC–MS/MS, MALDI-MS imaging and histopathology.

Table 4 Summary of average drug detection rates per study sample

<table>
<thead>
<tr>
<th>Biopsy sample ID</th>
<th>Biopsy type</th>
<th>Number of sections analysed</th>
<th>Number of sections with drug observed</th>
<th>% Success</th>
<th>Average % success for endobronchial samples</th>
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</thead>
<tbody>
<tr>
<td>4A</td>
<td>Transbronchial</td>
<td>25</td>
<td>5</td>
<td>20</td>
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<tr>
<td>4B</td>
<td>Endobronchial</td>
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<td>5</td>
<td>19</td>
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<tr>
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<td>Endobronchial</td>
<td>12</td>
<td>8</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>4D</td>
<td>Endobronchial</td>
<td>8</td>
<td>3</td>
<td>38</td>
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</tr>
<tr>
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<td>1</td>
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<td>4</td>
<td>4</td>
<td>100</td>
<td>90.0</td>
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<td>Transbronchial</td>
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<td>1</td>
<td>8</td>
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<td>Transbronchial</td>
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<td>0</td>
<td>0</td>
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</tr>
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<td>0†</td>
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<tr>
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<td>4</td>
<td>36</td>
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<td>Endobronchial</td>
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<td>14</td>
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</tr>
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<td>5</td>
<td>3</td>
<td>60</td>
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</table>

*Average success rate while incorporating sample 6E is 23.5%
† Due to issues we had with generating suitable sections from sample 6E for MALDI-MS imaging we have recalculated the average success for sample 6 excluding the data from biopsy E, updated result is 25%
A scaled preclinical study was initially conducted in rats to optimise assay conditions, due to the anticipated challenges with respect to the detection of a single clinical dose of ipratropium in relatively small lung biopsy samples. The preclinical study allowed sample handling methods and detection limits of ipratropium in rat lung samples (similar in size to human cryobiopsy samples) to be assessed by LC–MS/MS and MALDI-MS imaging. Widespread and even distribution of ipratropium was observed, see Additional file 1, in both rat lung sections and equivalent sized biopsies to those expected from TBC.

A total of seven participants were dosed with ipratropium bromide, with five participants providing both TBC and endobronchial samples. LC–MS/MS analysis demonstrated the presence of drug in all participants’ TBCs, suggesting that ipratropium was able to deposit in the distal lung, the area that is most affected in IPF.

Drug aerosol particle size by medical nebuliser is polydisperse, therefore containing a mix of different particle sizes. Particle size was not measured as we did not perform any drug delivery quantification. According to product literature the combination of a Porta Neb compressor (Phillips Respironics, Amsterdam, Netherlands) running at 6 L/min with a SideStream aerosolising chamber (Respironics, Tangmere, UK) achieves a mass median diameter of <5 µm in 80% of droplets generated. Salbutamol nebulised using the same compressor/nebuliser configuration gave a mean mass median aerodynamic diameter (MMAD) of 2.2 µm (SD 0.4) and a mean geometric standard deviation 3.45 µm (SD 1.1) [18]. Aerosol droplet size influences the location of particle deposition and alveolar deposition peaks at about 1.5 µm [19]. It was therefore reasonable to assume that the compressor/nebuliser configuration would create aerosol droplets of sufficiently small size to reach the target tissue. Due to insufficient TBC or endobronchial control material being available to prepare calibration standards no drug quantification measurements were made in this study.

Using LC–MS/MS requires homogenisation of the tissue hence results in a loss of anatomical and spatial information but allows the analysis of a larger sample and thereby can provide increased sensitivity. Conversely, MALDI-MS imaging provides spatial and regional information but is limited by the small sampling size. Due to the small sampling size used (typically 100 µm × 100 µm) achieving sufficient sensitivity in the clinical study proved difficult and LC–MS/MS analysis was used to confirm drug was present in biopsies. Although current MALDI-MS imaging sensitivity was generally unable to fully profile drug distribution in the TBCs, it was sufficiently sensitive to detect ipratropium in certain foci. The requirement for the coincident presence of both fragment ions in the MALDI-MS imaging data, at a signal to noise ratio threshold of 3:1 or greater as the threshold for the identification of ipratropium to be positively recorded as well as the fact that ipratropium was detected by LC–MS/MS in the remaining biopsy fraction for all distal lung biopsy samples, provides increased confidence that ipratropium was detected by MALDI-MS imaging. LC/MS was able to detect ipratropium in all distal lung biopsy samples. The sensitivity of MALDI MS is such that demonstrating colocalization with areas of histological fibrosis is more challenging. We were delighted to show this overlap in 75% of the fibrotic samples.

MALDI-MS imaging detected ipratropium in four participants’ TBC samples (Fig. 3), three of whom also had fibrotic regions identified within the TBC research samples. In some instances, e.g., Figure 4D (iii), the foci of ipratropium are not directly overlying the biopsy sample. This is likely due to diffusion/delocalisation of ipratropium from the periphery of the sample section during the sample freezing process within the embedding material and/or during the thaw-mounting process of the sample section onto the glass slide in preparation for MALDI-MS imaging. It is the authors’ opinion that this still constitutes the positive identification/detection of ipratropium in the sample section.

In all five participants, MALDI-MS imaging detected ipratropium in the endobronchial samples. More ipratropium foci and higher ipratropium signal intensities were detected in the proximal lung samples than distal lung samples even though proximal lung samples were smaller in size. This was expected, as in general inhaled drugs emitted from a device generating polydisperse particle sizes are more likely to deposit higher amounts of drug in the proximal lung and larger airways than the distal lung and alveoli [17]. In addition, with just a single dose of nebulised drug and only 10–30% of nominal dose expected to reach the lung (due to the efficiency of the nebuliser device) [20] and the estimated surface area of the human lung varying between 50 and 75 m² [21], it is expected to be challenging to detect drug deposited in 5 mm² distal lung TBC samples and if detected, would likely be close to the limit of the detection of any MALDI-MS imaging technique.

The deposition of an inhaled drug depends on the particle size distribution, inhaler device used and patient performance. In general, the nature of ILD may favour an inhaled drug approach. In fibrotic ILD the airways may be of wider calibre than normal due to airway splinting and distal traction bronchiectasis. In addition, FEV1 is preserved, and patients are usually able to generate reasonable inspiratory pressures required to use an inhaler. We observed minimal endobronchial secretions was at bronchoscopy to interfere with drug deposition which
contrasts to the situation in airways diseases, such as asthma, that may be complicated by mucus plugging.

Our study was performed using a monodisperse inhaler and other studies using aerosolised drugs have shown that smaller particles achieved greater total lung deposition (1.5 μm [56%], 3 μm [50%], and 6 μm [46%]), farther distal airways penetration (0.79, 0.60, and 0.36, respective penetration index), and more peripheral lung deposition (25, 17, and 10%, respectively) [22]. As well as nebulisers the other main types of inhaler devices are metered-dose inhalers (MDIs) and dry drug powder inhalers. Current inhalers generally have a broad particle distribution (0.5–6 μm), comparable to the nebuliser. The Respimat is a reusable soft mist MDI with a higher fine particle fraction (about 2.5-fold) and a slower velocity (× fivefold) compared to propellant-driven MDIs. It delivers approximately 60–70% of its dose in the respirable particle fraction (<1.0 μm) and is the only commercial device to deliver particles <0.3 μm. It is therefore likely that a soft mist MDI might allow delivery of drug even deeper into the lung, but this was beyond the scope of this study.

This proof of concept study studied a small number of patients, balancing risk of research biopsies against benefits of understanding inhaled drug distribution in fILD, and has several limitations. There was a difference between the demonstrated detection of ipratropium in the pre-clinical study versus the clinical study, despite using what was considered a scaled dose. The human ipratropium dose of 500 mcg was converted to 0.5 mcg/g in lung tissue by assuming a human lung weight of 1000 g. A similar assumption was made for rat lung weight of 1.5 g and the 0.5 mcg/g lung tissue dose was matched between the species. As this was an experimental study, we were not in a position to quantify the rat to human “disconnect”; we do not have systemic (plasma) data or quantified human lung concentrations. Indeed, the pre-clinical work was only performed to allow study sample workup and methodologies to be put in place. Possible explanations for the observed “disconnect” could be the effect of impaired lung function of the participants, pulmonary clearance mechanisms, or a degree of wash out of drug due to the administration of topical anaesthetic during the bronchoscopy. In the pre-clinical rat study, the lung levels for ipratropium appeared to be consistent throughout the 5–65 min time period. We assumed that this would be the same in humans, but this may not be the case. The delay of up to 60–70 min before biopsy may have contributed to some dissolution and absorption of the ipratropium in the airways. However, whilst topically active, ipratropium as a quaternary ammonium compound, is poorly absorbed [23] but has a reported short systemic half-life of 1.6 h [24]. Exact correlation with the underlying histopathology was sometimes confounded due to delocalization of drug, presumably during sample processing, together with limitations to the histological assessments resulting from the use of the embedding material and section thickness needed for sample preparation. While we were able to prove that inhaled ipratropium does deposit in distal, fibrosed lung in participants with ILD, we were not always able to show the exact location within the biopsy samples with confidence. As we were operating close to the limits of detection of the current instrument (MALDI), we could not show the potential drug distribution. Therefore, in further studies we would recommend use of an increase in drug dose and/or greater MALDI-MS sensitivity.

The advent of TBC has brought translational research opportunities by allowing minimally invasive and rapid access to lung interstitial tissue and therefore the potential to study relatively large distal lung biopsies without the need for a Video Assisted Thoracoscopic Surgery or open surgical approach. A further advantage over surgical acquisition of samples is the fact that participants are self-ventilating throughout the procedure which in this study should lead to a more physiological drug distribution than in ventilated participants. Time from nebulisation to biopsy is also reduced as the participant can be nebulised in the bronchoscopy suite directly before receiving sedation.

In this proof of concept study, we are able to present confirmation that inhaled drug therapy is a feasible route of administration for fibrotic ILD. However, further work is needed to encompass the influences of the varying physicochemical properties of different pharmaceutical formulations to be used in IPF to optimise distal delivery. Similarly, development of an inhaled therapy would also require an understanding and evaluation of drug clearance particularly since fibrotic interstitium between the alveolar epithelium and the blood supply would likely impair drug penetration into the blood vessels.

Future studies using this unique and the powerful combination of TBC and Mass Spectrometry have the potential to evaluate the ability of an inhaled, or systemic dosed molecule to reach the lung, and may in particular shorten the early clinical phase of an inhaled drug where target engagement is important to demonstrate early in development.

Conclusion

We have demonstrated in this study for the first-time using LC–MS/MS and MALDI-MS imaging that a drug taken via the inhaled route can deposit in distal fibrotic lung tissues. All participants had a fibrotic ILD with overall moderately impaired lung function. To our knowledge, this is the first study to directly assess the deposition of non-radiolabeled drugs to the distal lungs of participants
with ILDs and correlating histology with drug deposition in these participants. Ipratropium was detected in all TBC and endobronchial samples tested indicating that drug deposition reached the peripheral lung, a region that is most affected in IPF.

This study, therefore, in addition to the study by Usman et al., 2018 [17] suggests that ILD participants with established fibrosis can benefit from treatments administered by the inhaled route.

**Take home message**

Using mass spectrometry, this study demonstrates for the first-time that an inhaled drug can deposit in distal fibrotic lung parenchyma in patients. This finding suggests that drugs to treat pulmonary fibrosis could potentially be administered by the inhaled route.

**Abbreviations**

AEs: Adverse events; F: Fibrotic; H&E: Haematoxylin and eosin; ILD: Interstitial lung disease; IPD: Idiopathic pulmonary fibrosis; LC–MS/MS: Liquid chromatography—tandem mass spectrometry; MALDI–MS: Matrix assisted laser desorption ionisation—mass spectrometry; TBC: Transbronchial cryobiopsy.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12931-022-02026-5.

**Acknowledgements**

The authors would like to thank Christoph Nordmann at Bruker Daltonics, Bremen, Germany and Nigel Deeks (DMPK, GSK Research, UK) for their invaluable technical assistance.

**Author disclosures**

EO, MVB, JM, EJ, AC, RLT, PM are employees of GlaxoSmithKline (GSK) and hold shares/options. TAM, JCP, JS and RT were recipients of research funding by GlaxoSmithKline (GSK).

**Author contributions**

TAM, EO, EJ, AC, JM, PM and JCP designed the study. TAM, RT and JS performed the clinical study. PM and JM performed the MALDI–MS imaging and LC–MS/MS analysis. RLT performed histopathological analysis. EJ was the study statistician. TAM and PM drafted the manuscript. All authors read and approved the final manuscript.

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**EO:** GSK Scientist who co-designed the study, authored the protocol/study report and provided scientific insight of study.

**MVB:** GSK Early Development Leader for the fibrosis discovery performance unit at the time.

**RLT:** Pathologist working in Non-Clinical Safety, GSK UK.

**Funding**

This work was undertaken at University College London Hospital/University College London who received a proportion of funding from the Department of Health’s NIHR Biomedical Research Centre’s funding scheme. Funding for this study was also provided by GlaxoSmithKline (GSK Study ID: 205053; NCT: 03136120). JCP received funding as a Medical Research Council New Investigator and from Breathing Matters.

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Within 6 months of this publication, anonymized individual participant data, the annotated case report form, protocol, reporting and analysis plan, raw dataset, analysis-ready dataset and clinical study report will be available for research proposals approved by an independent review committee. Proposals should be submitted to www.clinicalstudydatarequest.com. A data access agreement will be required.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by London Camden and Kings Cross Research Ethics Committee and registered on clinicaltrials.gov (NCT03136120). All patients gave informed consent.

**Consent for publication**

Not applicable as no patient identifiable data.

**Competing interests**

The authors declare that they have no competing interests.

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Received: 14 May 2021 Accepted: 14 April 2022

**Published online:** 11 May 2022

**References**


Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Multi-center evaluation of baseline neutrophil-to-lymphocyte (NLR) ratio as an independent predictor of mortality and clinical risk stratifier in idiopathic pulmonary fibrosis

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Summary

Background Idiopathic pulmonary fibrosis (IPF) is a progressive, fatal disorder with a variable disease trajectory. The aim of this study was to assess the potential of neutrophil-to-lymphocyte ratio (NLR) to predict outcomes in IPF.

Methods We adopted a two-stage discovery (n = 71) and validation (n = 134) design using patients from the UCL partners (UCLp) cohort. We then combined discovery and validation cohorts and included an additional 794 people with IPF, using real-life data from 5 other UK centers, to give a combined cohort of 999 patients. Data were collected from patients presenting over a 13-year period (2006–2019) with mean follow up of 3.7 years (censoring: 2018–2020).

Findings In the discovery analysis, we showed that high values of NLR (> / = 2.9 vs < 2.9) were associated with increased risk of mortality in IPF (HR 2.04, 95% CI 1.09–3.81, n = 71, p = 0.025). This was confirmed in the validation (HR 1.91, 95% CI 1.15–3.18, n = 134, p = 0.0114) and combined cohorts (HR 1.65, n = 999, 95% CI 1.39–1.95; p < 0.0001). NLR correlated with GAP stage and GAP index (p < 0.0001). Stratifying patients by NLR category (low/high) showed significant differences in survival for GAP stage 2 (p < 0.0001), however not for GAP stage 1 or 3. In a multivariate analysis, a high NLR was an independent predictor of mortality/progression after adjustment for individual GAP components and steroid/anti-fibrotic use (p < 0.03). Furthermore, incorporation of baseline NLR in a modified GAP-stage/index, GAP–index/stage-plus, refined prognostic ability as measured by concordance (C)-index.

Interpretation We have identified NLR as a widely available test that significantly correlates with lung function, can predict outcomes in IPF and refines cohort staging with GAP. NLR may allow timely prioritisation of at-risk patients, even in the absence of lung function.

Funding Breathing Matters, GSK, CF Trust, BLF-Asthma, MRC, NIHR Alpha-1 Foundation.

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Articles

Evidence before this study
There is an urgent need for biomarkers to better stratify patients with idiopathic pulmonary fibrosis (IPF) for clinical trials and transplant allocation. We investigated whether the neutrophil-to-lymphocyte ratio (NLR) in the peripheral blood could refine the current clinical scoring system (GAP: gender, age, and physiology) to identify cohorts of patients with IPF at higher risk of poor outcomes. We searched the scientific literature using PubMed to identify studies in which the baseline NLR had been used to predict outcomes for patients with IPF. We used the search terms “IPF”, “pulmonary fibrosis” and “NLR” and did not use language or date restrictions. We identified seven studies that specifically considered NLR as a biomarker in IPF. Of these two were small single centre studies and a third study measured NLR in bronchoalveolar lavage (BAL). Nathan et al. included 1334 patients with IPF from ASCEND (Study 016; NCT01366209) and CAPACITY (Studies 004 and 006; NCT00287716 and NCT00287729) as a discovery cohort and placebo-treated IPF patients from two independent Phase III, trials of IFN-γ-1b (GIPF-001 (NCT00047645) and GIPF-007 (NCT00075998) as a validation cohort. Finally, the most recent study compared the predictive potential of NLR in fibrosing hypersensitivity pneumonitis (HP) compared with IPF. None of these studies validated the ability of NLR to predict mortality beyond 12 months in IPF and there is no data on the incorporation of NLR in an adjusted GAP score.

Introduction
Idiopathic pulmonary fibrosis (IPF) is a progressive, fatal disorder with a very variable disease trajectory. Available treatments for IPF are expensive and merely slow disease progression with frequent side-effects. A prognostic biomarker would guide treatment decisions, timing of lung transplant or end of life care and help patients and clinicians to plan.

Clinical cohort staging in IPF relies on the Gender, Age, Physiology (GAP) index (a score from 0 to 7) with associated GAP stage (I-III), a static measure unable to identify rapidly deteriorating patients, or assess treatment response. There is an unmet need for biomarkers to guide a personalized approach to care, as well as for cohort stratification in clinical trials. Only two biomarkers have been validated to refine the GAP staging system by identifying high and low risk patients within a given GAP stage. The first used a 52-gene expression signature, an approach that requires calibration against a control cohort, and the second measured glucose uptake in the lung with Positron Emission Tomography (PET).

Both biomarkers require specialist expertise and are costly, limiting their practicality. The ideal biomarker would be measurable in the blood using a simple and widely available test and would predict prognosis and potentially response to therapy.

The Neutrophil-Lymphocyte Ratio (NLR) is easily and inexpensively measured from a complete blood count (CBC), and has indicated severity in studies of diabetes, cardiovascular and renal disease, COPD, malignancy and COVID-19. NLR can also predict development and severity of lung fibrosis in patients with systemic sclerosis, dermatomyositis/polymyositis and a composite endpoint of ‘absolute decline in 6MWD ≥50 m or death’ at 12 months in IPF. It is not known whether addition of NLR to GAP will
refine clinical cohort staging in IPF and guide management.

Here we use a two-stage derivation and validation model to determine a discriminatory cut-off for low (<2.9) or high (≥2.9) NLR. We combined our derivation and validation cohorts with additional external cohorts to give a combined cohort of 999 patients to then investigate the ability of NLR to refine the prognostic power of the clinical GAP score in IPF.

Methods
Study design and participants
An observational study to evaluate NLR as an independent mortality risk predictor in IPF. Derivation cohort, 71 IPF patients enrolled at UCLH (2008–18). Additional cohort of 928 patients comprising: Validation cohort for NLR of 134 patients from UCLH (2006–18); External cohorts of 794: 279 IPF patients from Royal Brompton and Harefield NHS Trust (RBH) (2006–2018); cohort of 515 IPF patients from Southwest and Leicester (SW&L): Royal Devon and Exeter (RDE) Hospital (300), North Bristol (NB) NHS Trust (85), Taunton and Somerset (TS) NHS Foundation Trust (30); and University Hospitals of Leicester (UHL; 90), (2011–2019). Total combined cohort, N = 999. Inclusion criteria: diagnosis of IPF; baseline pulmonary function tests and CBC. Exclusions: malignancy or haematological disorder; infection at time of CBC (CRP >20 mg/L, clinical/ imaging signs of infection); cytotoxic drugs. Exclusion from derivation cohort if on prednisolone >5 mg or equivalent at time of diagnosis and analysed according to local NHS protocols. There was no standardization of analysis, or of normal ranges, between sites. An inclusion level of CRP <20 mg/L was chosen as shown to be discriminatory for excluding bacterial infections in adults11.

Antifibrotic data was available for the Southwest cohorts (RD&E, MPH, NBT) and RBH for patients on antifibrotics for >6 weeks. However, neither time nor duration of therapy, was recorded.

We have previously reported part of our UCLH internal derivation and validation cohorts, 208 patients, as an abstract.14 The 515 patients from NB/RDE/TS/UHL were reported as part of a larger cohort comparing basic outcome predictors in IPF versus fHP15.

Outcomes
Primary outcome measure was transplant free survival from CBC measurement to death (all-causes) or transplant in high and low NLR groups using the following censoring: UCLH 28/6/2018, RBH 30/1/2020, SW&L 12/7/2019. Secondary outcome was assessment of NLR as a mortality predictor in comparison to GAP index-predicted mortality (Table S1)16 and independence of GAP index.

Statistical analysis
A non-biased empirical Cumulative Distribution Function, cCDF plot of baseline NLR of the derivation cohort was used to determine the median NLR. Harrell’s concordance (C)-index was used to determine the ability of NLR to predict outcome accurately with increasing time from baseline. Different models are compared by C-index with an increase in the C-index indicating an improvement in the model. Analysis was performed using STATA 15 (Stata Corp, College Station, Texas). Fisher’s exact test and unpaired tailed t-tests were used to calculate significance between different group characteristics. Although a normal distribution of data was not formally proven, histograms of lung function, age, and GAP index between high and low NLR groups, were not observed to be skewed and with no extreme outliers, and, given the large sample size, the application of the t-test was acceptable.27 Further sensitivity analysis was performed using non-parametric tests. All p-values are reported for two-sided confidence intervals. A p-value of <0.05 was considered significant. However, as C-indices are not sensitive enough to detect statistical differences between models, p values for differences in C-indices are not reported.

Survival analysis
Both transplant and death were events. Univariate analysis was used to calculate risk of death/prediction of transplant-free survival and the relationship between NLR, NLR category (high/low), GAP Index, GAP Stage, age, sex, FVC (% predicted), TLco (% predicted), steroid therapy (as a binary variable), and transplant-free survival. Significance testing between groups on Kaplan–Meier curves was performed using non-parametric log-rank test. The log rank test was used to test the null hypothesis that there is no difference in survival between pre-specified groups (such as high vs low NLR). The ‘expected’ failure rates are what would be expected for each group if there was no difference in survival between the two, the ‘observed’ are the actual rates. Multivariate stepwise forward cox proportional hazards regression was used to determine whether NLR (as a continuous parameter or category) was independent of the GAP index/stage (and their individual components) and steroids in predicting patient transplant-free survival.

GAP Index-Plus and GAP Stage-Plus: For the NLR-modified GAP calculation, we proposed adding a fourth NLR variable that was binarized, as high (≥2.9)/adverse (coded as 1) or favorable (<2.9) (coded as 0). This was then added to the existing GAP Index calculation where the modified GAP Index ranged from 0 to 9. For example, if a patient with original GAP Index “0” had a high NLR the modified GAP Index would be “0 + 1” = “1”. Conversely, if the patient with original GAP index “0” had a low NLR the modified GAP index would be “0 + 0” = “0”. So the “new” modified GAP
index, which we called GAP Index-Plus ranged from 0 to 9 in comparison to the original GAP Index, which ranged from 0 to 8.

For GAP Stage-Plus we up-staged patients’ GAP stage by 1 if they were in the high NLR category. In this way we had a four category GAP stage such that original GAP Index of 0–3 = Stage 1; GAP Index of 0–3 plus high NLR = Stage 2; GAP Index of 4–5 = Stage 2; GAP Index of 4–5 plus high NLR = Stage 3; GAP Index of 6–8 = Stage 3; GAP Index of 6–8 plus high NLR = Stage 4.

The decision to have two different modifications that are not interchangeable was for ease of use for calculating GAP Stage-Plus or Index-Plus dependent on low (+0) or high (+1) NLR, as original GAP Index and Stage are both easily calculated by many available smartphone applications.

Ethical approvals

Ethical approval was granted by the HRA and Health and Care Research Wales (HCRW) (REC reference: 18/LO/0937). Site specific and local R&D approvals were obtained at each participating site. Informed consent was not required for this anonymised, retrospective data.

Role of the funding source

The funders had not input into the study design or interpretation. Access to the data set is available by contacting the corresponding author. The decision to submit the manuscript was made by JCP and TAM with agreement from all other authors.

Results

Patient characteristics in the individual and pooled cohorts are summarised in Table 1 for demographic data available across the whole dataset. Data was not available across the whole data set for ethnicity, smoking status, BMI or other co-morbidities. For the 999 patients in the combined (discovery, validation and additional) cohorts, there were 533 events (death or transplant) recorded.

The median NLR in the derivation cohort was 2.9 (95% CI, 2.2–4.1) and we used this cut-off to determine high (/> = 2.9) or low NLR (<2.9). Median NLR across the additional cohorts was similar with UCLH validation cohort, 3.1 (2.0–4.4), Exeter additional cohorts 2.8 (2.1–4.0), and RBH additional cohort 3.2 (2.3–4.8). The combined cohort of 999 patients had a median NLR of 2.9 (2.1–2.3). When the original NLR cut-off of 2.9 was applied to the combined cohort, increasing age, male sex, and worse lung function parameters were all associated with the high NLR category (Table 2).

For the derivation cohort (n = 71) there was a significant difference in the median survival between high NLR (> = 2.9) or low NLR (<2.9) with median survival of 62.1 months (IQR 20.2–na), in the low NLR group (n = 36), versus 24.3 months (IQR 11.4–69.8) in high NLR (n = 35), p = 0.0125. This increased mortality was confirmed in the validation cohort (n = 134) with median survival in the low NLR group (n = 64) of 46.5 months (IQR 16.8–93.50) and in the high NLR group (n = 70) of 16.9 months (IQR 9.7–43.4), p = 0.0125 (Table 3).

For each of the individual external cohorts the improved survival with low NLR was consistent (Table 3). SW&L: median survival low NLR (n = 297) of 57 months (IQR 32–157), high NLR (n = 218) of 44 months (IQR 18–121) p = 0.0037; RBH cohort, median survival low NLR (n = 120) of 46.5 months (IQR 22.6–80), high NLR (n = 159) of 39.8 months (IQR 19.8–58.8) p = 0.0223 (Table 3). The same was seen for the combined validation and external cohorts (n = 928 (excluding discovery), median survival low NLR (n = 466) of 49.6 months (IQR 25.9–89.5), high NLR (n = 462) of 39 months (IQR 16–63.7) p<0.0001.

Finally, the patients were taken as a combined cohort of 999 and were divided into high NLR (> = 2.9) or low NLR (<2.9) at time 0; there was a significant difference in the median survival between high and low NLR groups (Fig. 1; p < 0.0001). Median survival in the low NLR group (n = 502) of 49.8 months (IQR 24.8–88.3), incident rate of 0.013 and a total time at risk of 17,707 months; median survival in the high NLR group (n = 497) of 35.9 months (IQR 15.1–63.7), incident rate of 0.021 and a total time at risk of 14,426 months (Table 3 and Fig. 1).

We then used this combined cohort of 999 patients, to investigate whether the addition of NLR could refine the GAP clinical scoring. We first showed that the association of NLR category with GAP stage or GAP Index was highly significant (Table 2; p < 0.0001). Although gas transfer data was only available for 71% of subjects, a lower TLco was significantly associated with high NLR (42.2% pred. versus 47.7%, p < 0.0001).

For this combined patient cohort, the observed mortality was similar to that predicted using GAP stage predicted mortality*: (Supplementary Table S1). Median survival per GAP stage is summarised in Table 4. Median survival as stratified by NLR risk category was not significantly different for GAP stage 1 (p = 0.245) or 3 (p = 0.1381) but was significant for GAP stage 2 (p = 0.0127) and for the remaining patients who had no GAP stage recorded due to insufficient lung function data (p = 0.0015; Table 5).

The difference in expected versus observed events, based on log-rank test for equality of survivor functions, for patients in high and low NLR categories in the combined cohort (n = 999) was significant with 235 observed events out of 300.95 expected in the low NLR group, versus 303 observed events out of 237.05 expected in the high NLR group (log rank test, p < 0.0001; Fig. 1).
Differences between survival in patients with different GAP stages 1–3 (Fig. 2A) and GAP Index scores (not shown) reached significance (log rank test, p < 0.0001). Stratifying patients in the same GAP stage by NLR category (low/high) only showed significant differences in survival between low and high NLR for GAP stage 2 (log rank test, p < 0.0001; Fig. 2C), and not for GAP stage 1 (log rank test, p = 0.1755; Fig. 2B), or stage 3 (log rank test, p = 0.0871; Fig. 2D).

We proposed an NLR-modified GAP calculation, GAP Index-plus and GAP Stage-Plus (see methods) using a very simple modification of GAP dependent on low (+0) or high (+1) NLR, which was memorable and easily used. Survival differences between groups were

<table>
<thead>
<tr>
<th>Derivation cohort n = 71</th>
<th>Internal validation cohort n = 134</th>
<th>External Additional Exeter (all sites) n = 515</th>
<th>External Additional RBH n = 279</th>
<th>Combined all cohorts N = 999</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years (SD)</td>
<td>71.05 (9.0)</td>
<td>74.7 (8.6)</td>
<td>74.0 (8.6)</td>
<td>69.6 (8.7)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>62 (87.3%)</td>
<td>107 (79.9%)</td>
<td>380 (73.8%)</td>
<td>219 (78.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>9 (12.7%)</td>
<td>27 (20.2%)</td>
<td>135 (26.2%)</td>
<td>60 (21.5%)</td>
</tr>
<tr>
<td>Lung function*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC (%) (SD)</td>
<td>77.8 (17.9) n = 70</td>
<td>77.1 (21.1) n = 133</td>
<td>82.6 (20.2) n = 362</td>
<td>72.9 (16.6) n = 278</td>
</tr>
<tr>
<td>FEV1 (%) (SD)</td>
<td>78.0 (16.4) n = 69</td>
<td>82.5 (22.3) n = 123</td>
<td>86.0 (20.7) n = 299</td>
<td>77.2 (16.6) n = 266</td>
</tr>
<tr>
<td>Tlco (%) (SD)</td>
<td>44.8 (13.9) n = 61</td>
<td>47.9 (17.9) n = 114</td>
<td>49.9 (15.7) n = 274</td>
<td>41.9 (14.2) n = 258</td>
</tr>
<tr>
<td>Tlco unable</td>
<td>8</td>
<td>12</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Tlco not done/recorded</td>
<td>2</td>
<td>8</td>
<td>240</td>
<td>0</td>
</tr>
<tr>
<td>GAP index mean (SD)</td>
<td>4.2 (1.6) n = 69</td>
<td>4.4 (1.5) n = 126</td>
<td>3.8 (1.3) n = 275</td>
<td>4.3 (1.4) n = 279</td>
</tr>
<tr>
<td>GAP stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20 (29.0%)</td>
<td>34 (27.0%)</td>
<td>124 (45.1%)</td>
<td>77 (27.6%)</td>
</tr>
<tr>
<td>2</td>
<td>36 (52.2%)</td>
<td>65 (51.6%)</td>
<td>124 (45.1%)</td>
<td>143 (51.3%)</td>
</tr>
<tr>
<td>3</td>
<td>13 (18.8%)</td>
<td>27 (21.4%)</td>
<td>27 (9.8%)</td>
<td>59 (21.2%)</td>
</tr>
<tr>
<td>NLR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3.9 (3.3)</td>
<td>4.1 (3.6)</td>
<td>3.5 (2.8)</td>
<td>4.6 (4.2)</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>2.9 (2.2-4.1)</td>
<td>3.1 (2.0-4.4)</td>
<td>2.8 (2.1-4.0)</td>
<td>3.2 (2.3-4.8)</td>
</tr>
</tbody>
</table>

*p values were calculated by unpaired two-tailed t-test except for sex and GAP stage where a Fisher’s exact test was used.

Table 1: Baseline characteristics of the patients (combined cohort, n = 999) in low (<2.9, n = 502) and high (≥2.9, n = 497) NLR risk groups.
significant for both GAP Index-plus (HR 1.4, 95% CI 1.29–1.51, p < 0.0001; figure not shown) with survival differences between the GAP Stage-plus groups of patients also reaching significance (HR 1.80, 95% CI 1.60–1.98; log rank test, p < 0.0001; Fig. 3).

Univariate Cox proportional hazards models of the combined cohort (n = 999) showed that patients in the high NLR category group had significantly higher mortality/progression to lung transplant when compared with patients in the low NLR group (HR 1.65, 95% CI 1.39–1.95; p < 0.0001; not shown), reflecting their baseline demographics (Table 2). NLR category remained significant when each site’s cohort was considered individually (Fig. 4). Analysis was repeated in the combined cohort excluding all patients on known steroid therapy with a comparable result (HR 1.50, 95% CI 1.24–1.82; p < 0.0001; data not shown). Univariate regressions for GAP Index, GAP Stage, GAP Index-plus and GAP Stage-plus were all significant (GAP index, HR 1.4, 95% CI 1.3–1.5, p < 0.0001; GAP Stage, HR 2.1, 95% CI 1.8–2.4, p < 0.0001; GAP Index-plus, HR 1.4, 95% CI 1.29–1.51, p < 0.0001; GAP Stage-plus HR 1.8, 95% CI 1.6–2.0, p < 0.0001). Univariate regression was carried out for all the individual GAP components (age, sex, FVC % pred, TLco % pred) and was significant for all except sex. Age, HR 1.02, 95% CI 1.1–1.03, p < 0.0001; sex, HR 1.2, 95% CI 0.99–1.51, p = 0.065; FVC% pred, HR 0.98, 95% CI 0.97–0.99, p < 0.0001; TLco% pred, HR 0.97, 95% CI 0.96–0.97, p < 0.0001.

There was significant difference in the individual components of GAP (age, FVC% pred, TLco% pred but not gender) between patients with high and low NLR based on non-parametric Wilcoxon rank-sum test (Age, p = 0.003; FVC% pred, p = 0.0023; and TLco% pred p < 0.0001). Cox regression for steroid use was also significant for transplant-free survival (HR 1.71, 95% CI 1.37–2.12 p < 0.0001). The analysis was then repeated in this cohort but with the exclusion of all those patients who had ever taken oral steroids and showed the same significances.

Multivariate analysis was then performed using these individual components as covariates within the model: age, sex, FVC%, TLco%, GAP Stage, use of steroids, NLR (continuous or binary high/low). This analysis showed that after adjusting for GAP Stage and use of steroids in the combined dataset a high NLR category remained independently predictive of mortality/progression to lung transplant (HR 1.36, 95% CI 1.12–1.66; p = 0.002). Repeating the analysis using the individual GAP components (age, sex, FVC%, TLco%) as variables and again adjusting for steroids showed similar results (HR 1.26, 95% CI 1.03–1.55; p = 0.027). Inputting NLR as a continuous variable was also independently predictive adjusted for the individual GAP components and steroids (HR 1.04, 95% CI 1.01–1.07; p = 0.011) as well as when adjusted for GAP stage and steroid use (HR 1.05, 95% CI 1.02–1.07, p = 0.001). All GAP

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**Table 3: Median survival in low (<2.9, n = 502) and high (>2.9, n = 497) NLR risk groups per study site/ cohort**

<table>
<thead>
<tr>
<th>Site/Cohort</th>
<th>Low NLR</th>
<th>Median survival months (IQR)</th>
<th>Incident rate</th>
<th>Total time at risk (months)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Derivation cohort</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCLH n = 71</td>
<td>n = 36</td>
<td>0.0003</td>
<td>975.6</td>
<td>0.026</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Internal validation</td>
<td>n = 64</td>
<td>0.017</td>
<td>1226.5</td>
<td>0.031</td>
<td>0.021</td>
</tr>
<tr>
<td>cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>External additional</td>
<td>n = 297</td>
<td>0.016</td>
<td>5479</td>
<td>0.023</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SW&amp;L n = 515</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>External additional</td>
<td>n = 120</td>
<td>0.017</td>
<td>6356.6</td>
<td>0.021</td>
<td>0.021</td>
</tr>
<tr>
<td>plus external</td>
<td>n = 466</td>
<td>0.023</td>
<td>13450.8</td>
<td>0.021</td>
<td>0.021</td>
</tr>
<tr>
<td><strong>Combined Cohort</strong></td>
<td>N = 999</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCLH validation</td>
<td>n = 466</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plus external</td>
<td>N = 502</td>
<td></td>
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</tr>
</tbody>
</table>

**Fig. 1: Kaplan-Meier survival curve for all-cause mortality following a diagnosis of IPF for patients in low (<2.9, n = 502) and high (>2.9, n = 497) NLR category at baseline, with follow up of 40 months.** The numbers of patients at risk at 10, 20, 30, 40 months for each of these groups is shown in the table immediately below the survival curves. This demonstrates a significant difference in mortality between high and low categories (log rank test, p < 0.0001).
components, except for sex, continued to be significant when adjusted for each other, NLR and steroid use.

Although, for most of the patients, the baseline CBC predated the use of anti-fibrotics, some patients were later started on anti-fibrotics. Patients who had taken anti-fibrotics for >6 weeks were identified in the Southwest (RD&E, MPH, NBT; n = 415) and RBH (n = 270) cohorts. In Southwest cohort 275 of 415 (66.3%), and in RBH cohort 231 of 270 (85.6%) patients were recorded to have taken anti-fibrotics. Therefore, of a sub-cohort of 685 patients, 606 (73.9%) had taken anti-fibrotics. Univariate regression for anti-fibrotic therapy was not significant for mortality (HR 1.01, 95% CI 0.79–1.29, p = 0.95). Multivariate regression taking into consideration NLR category and anti-fibrotic therapy showed that anti-fibrotic use remained a non-significant predictor (HR 1.02, therapy 95% CI 0.79–1.30, p = 0.899), whereas NLR category was significant (HR 1.59, 95% CI 1.30–1.95, p < 0.0001).

Harrell’s concordance (C)-index prediction accuracy conrmed that the best performing prediction model was based on the component variables making up the GAP, with NLR as a continuous variable and adjusted for steroids. GAP index was clearly better than GAP stage, but incorporating NLR into GAP staging as GAP Index-Plus further increased the model’s ability to predict patient mortality (C-index 0.673, 0.645–0.701; Table 6).

Time-dependent ROC analysis in the pooled cohort for NLR demonstrated the continuous decline of the model’s predictive value with the passing of time. For example, AUC at 6 months is 0.728 which declines to an AUC of 0.598 at 48 months (Fig. 5).

**Discussion**

IPF is a devastating disease with a variable clinical course. One of the most used prognostic cohort scoring systems is the GAP score. However, even within the same GAP stage, and particularly for moderately severe GAP stage II, patients may have very heterogeneous outcomes. This has led to a concerted effort to identify better tools for individual patient risk stratification. The ideal biomarker would be measurable in the serum using a simple and widely available test and would predict prognosis and potentially response to therapy. Here we show that baseline NLR derived from a cheap and widely available routine blood test, identifies two groups of patients with IPF with significant differences in outcome. We go on to show that NLR can significantly refine the predictive capacity of the clinical GAP index.
The search for viable biomarkers has taken advantage of the rapidly expanding knowledge of IPF immunopathogenesis. Aberrant repair processes initiated by repetitive injury to the alveolar epithelium result in an exaggerated tissue remodelling response and fibrosis of the lung parenchyma. Proteins released from damaged epithelium and collagen degradation products can enter the systemic circulation, acting as markers of disease activity by proxy—the most promising of which include CA-19-9, CA-125 and CCL18. Others that have been investigated include SP-D, MMP7, osteopontin (OPN), periostin (PON), ICAM1 and telomere length. In addition, neo-epitopes generated by the action of matrix metalloproteinases (MMPs) on collagen can be detected in the serum and Jenkins et al. found that 6 of 12 of these were predictive for mortality. Other serum biomarkers include CD28, ICOS, LCK, ITK alone or as part of a 52-gene RNA signature. More recently, attention has turned to imaging biomarkers including imaging quantification, measurements of glucose uptake in the lung with Positron Emission Tomography (PET) and a combination of the two.

However, of these, only three biomarkers have been validated to refine the GAP staging system by identifying high and low risk patients within a given GAP stage. The 52-gene expression signature, an approach that requires calibration against a control cohort, a composite of OPN, PON, MMP-7 and ICAM-1 and the Total-to-Background Ratio (TBR) calculated from 18F-FDG-PET imaging, with only the 52-gene expression validated in multiple independent cohorts.

Fig. 2: Kaplan-Meier survival curves for all-cause mortality following a diagnosis of IPF with follow up extending to 40 months: A, All patients in combined cohort (n = 999) divided into GAP stages 1 (n = 255), 2 (n = 368) and 3 (n = 136); B, Patients in GAP Stage 1 stratified into low (<2.9, n = 154) and high (≥2.9, n = 101) NLR category at baseline; C, Patients in GAP Stage 2 stratified into low (<2.9, n = 170) and high (≥2.9, n = 198) NLR category at baseline; D, Patients in GAP Stage 3 stratified into low (<2.9, n = 48) and high (≥2.9, n = 78) NLR category at baseline. The numbers of patients at risk at 10, 20, 30, 40 months for each of these groups is shown in the table immediately below the survival curves. Differences between survival in patients with different GAP stages 1–3 (A) reached significance (log rank test, p < 0.0001). Stratifying patients in the same GAP stage by NLR category (low/high) only showed significant differences in survival between low and high NLR for GAP stage 2 (log rank test, p < 0.0001; (C)), and not for GAP stage 1 (log rank test, p = 0.1755; (B)), or stage 3 (log rank test, p = 0.0871; (D)).
NLR which is calculated from complete blood count with differential, is an inexpensive, easy to obtain, widely available and emerging marker of disease activity and prognosis in patients with chronic inflammatory diseases, cardiovascular diseases, and malignancies.

Previous studies have specifically considered NLR as a biomarker in IPF: Of two small single centre studies; the first\(^1^\) found NLR raised in 21 patients with IPF compared to 42 healthy controls but was not prognostic; the second study of 73 patients with IPF and 62 healthy controls found that NLR and monocyte lymphocyte ratio (MLR), but not platelet to lymphocyte ratio (PLR), associated with IPF and correlated negatively with FVC/TLco.\(^2^\) Another study measured NLR in bronchoalveolar lavage (BAL) samples from 59 patients with IPF and found that BAL NLR was inversely correlated with FVC/TLco.\(^3^\) We presented our discovery and validation cohort of 218 patients in 2018.\(^4^\) Our initial findings were taken further by Nathan et al.,\(^5^\) who included 1334 patients involved in ASCEND (Study 016; NCT01366209) and CAPACITY (Studies 004 and 006; NCT00287716 and NCT00287729) as a discovery cohort and placebo-treated IPF patients from two independent Phase III, trials of IFN-γ-1b (GIPF-001 (NCT00047645) and GIPF-007 (NCT00075998) as a validation cohort. Significant trends were observed between baseline NLR and PLR quartiles for various outcomes including: physiological decline; respiratory hospitalization; and all-cause mortality. However, the only consistent correlation in the discovery cohort was with baseline NLR and the composite endpoint of ‘absolute decline in 6MWD ≥50 m or death’ at 12 months, a finding that was not tested against the validation cohort. Alongside this other groups were investigating circulating cellular biomarkers in IPF. Significant prognostic effects were found for monocyte count a finding validated in >7000 patients with IPF from five independent cohorts\(^6^\) and >2000 patients from a further four cohorts.\(^7^\) However, the ability of monocyte count to enhance the predictive accuracy of GAP, although promising has not been validated\(^8^\) in clinical cohorts.

In this retrospective study, we have extended the findings of Nathan et al. to investigate the role of NLR in multiple ‘real-life’ IPF cohorts with a longer follow-up period, to see if the current clinical prediction GAP score could be further refined. We analysed the NLR in a derivation cohort of patients and identified a median value of NLR that separated our discovery population into a high and low risk group for transplant-free survival with significant differences in mortality. We then investigated the prognostic ability of this NLR cut-off in an internal validation cohort of IPF patients and then in a combined cohort which included the addition of two further IPF cohorts provided by five other ILD specialist service centers in the UK. Furthermore, we showed, using a variety of statistical models, that the NLR is an independent risk factor for mortality, and addition of NLR risk profiles to further refine GAP index cohorts significantly increased the prediction accuracy of this clinical score. Although NLR is, unsurprisingly, even more highly predictive as a continuous measure than as a binary ‘high’ or ‘low’, our aim was to modify the GAP score in a simple memorable way, and so we opted for a simple modification of GAP (+1 for ‘high’; +0 for ‘low’) rather than to create a complex composite score in which absolute NLR is incorporated into GAP.

We went on to show that the addition of NLR data to GAP score refines the existing mortality prediction model by using C-index and ROC statistics. As expected, the more granular the data inputted the better the prediction model, hence the increased C-Index for a model using the individual components of the GAP Index rather than an overall score. This despite marked heterogeneity between the cohorts, with the SW&L cohort being more recent (2011–2019) with a lower average GAP Index, GAP Stage and mean, and median NLR compared with the other cohorts. It is encouraging that NLR mortality prediction was robust despite this heterogeneity, pointing to widespread applicability.

However, we should emphasise that although the use of GAP scoring with the addition of the binary high/low NLR provides an easily applied tool to establish clinical cohorts of patients; GAP/NLR, although an improvement on GAP alone, is still limited when used for individual, rather than cohort, prognostication. The C-index, although improved is still only 0.71 which is
lower than other biomarkers used for clinical decision making. A more robust approach for an individual patient would ultimately necessitate input of more granular data, an approach that underlies scoring systems such as the composite physiological index (CPI).

By using time-dependent ROC analysis we were able to calculate the decline in NLR’s predictive accuracy over time and establish that it is most accurate shortly after being measured, a time when indeed it is most useful. Many newly diagnosed patients are keen to discuss prognosis early and as clinicians we often refer to variable disease trajectory and the need to observe lung function over time to allow more accurate prognostication. However, these data suggest that even early mortality might be predictable from a high NLR at presentation and may expedite, for example, lung transplant assessment in appropriate patients. A similar decline in predictive accuracy with time has been shown for GAP and other biomarkers.

The difference in median survival stratified by GAP stage was only significant for patients in the moderately severe GAP stage 2 (n = 368) and in those patients in whom the GAP stage could not be calculated (n = 250). This probably reflects the small number of events for patients at GAP stage 1 and the small number of patients at GAP stage 3 (n = 126), although similar trends to significance in these groups are encouraging. In those patients in whom GAP could not be calculated it was interesting to observe that the overall median survival of 60 months was between the median survivals for GAP Stages 1 (73.7 months) and GAP Stage 2 (41 months). When the patients in GAP Stage 2 were stratified according to NLR category, a remarkable difference of survival became apparent on either side of this median with Gap Stage 2 and low NLR having a median survival of 83 months, and almost double that of those in GAP Stage 2 with a high NLR whose median survival was just 44 months. Although less helpful for...
mild (stage 1) or very ill (stage 3) patients, the ability of a combined NLR/GAP score to further refine those moderately severe IPF patients (stage 2), is particularly helpful as: stage 2 is the more frequently represented stage; outcomes of stage 2 are the most heterogenous and it is in this patient group that clinical decision making can be most difficult.

GAP staging was not possible for those patients with incomplete lung function data, nearly always due to missing TLco readings. Gas transfer may be missing for several reasons, either the patient is unable to perform the test hence coded as a “3” (maximum) in the GAP index or a data quality issue. We found that patients with no TLco but in the low NLR group had a longer median survival than other patients in GAP stage 1, indicating that this subgroup may have been too well to warrant full lung function work up at the time of presentation. One additional feature of this study is our demonstration that NLR correlates with lung function, suggesting NLR may offer a cheap and quick screening test to fast-track high risk patients for early tertiary care review and urgent lung function. In fact, NLR as a continuous variable was almost as predictive as GAP score (Table 6: C-index of 0.66 for GAP Index versus 0.61 for NLR) and potentially easier to generate as there is no reliance on lung function.

Table 6: Harrell’s C-index performance in the pooled patient cohort (n = 999) including steroid users in various predictive models.

### Cox regression model

<table>
<thead>
<tr>
<th>C-index 95% CI</th>
<th>Cox regression model</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><strong>UNIVARIATE</strong></td>
</tr>
<tr>
<td></td>
<td>GAP Stage</td>
</tr>
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<td></td>
<td>GAP Index</td>
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<td></td>
<td>NLR Category</td>
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<td></td>
<td>GAP Stage Plus</td>
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<td>GAP Index Plus</td>
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<td><strong>MULTIVARIATE</strong></td>
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<td></td>
<td>GAP Stage</td>
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<td></td>
<td>NLR category</td>
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<td></td>
<td>GAP Stage</td>
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<tr>
<td></td>
<td>NLR, age, sex, FVC% predict, TLCO% predict, steroids</td>
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<tr>
<td></td>
<td>NLR category, age, sex, FVC% predict, TLCO% predict, steroids</td>
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<tr>
<td></td>
<td>NLR category, age, sex</td>
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<td>Age, sex</td>
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Fig. 5: Ability of baseline NLR to predict all-cause mortality in patients with IPF decreases with time. Time dependent change of AUC and ROC in NLR at 6, 12, 18, 24, 30, 36, and 42 months: AUC, area under the curve; ROC, receiver operator characteristic.
pandemic and in remote, and resource poor, areas where access to lung function is restricted. In addition, lung function can be influenced by operator, equipment, and patient factors such as sub-optimal maneuvers whereas CBC analysis maintains objectivity.

It is unclear why NLR is raised in patients with IPF with decreased survival. We propose it could be a marker for ongoing inflammation. The term interstitial lung disease or ILD covers a group of over 200 different diseases with varying degrees of inflammation and/or fibrosis. It is unclear whether fibrosis is always preceded by inflammation, although this is more likely to be true for ILDs associated with underlying autoimmune rheumatic diseases. In such situations, NLR has been shown to predict development and extent of lung fibrosis, for example in systemic sclerosis, and dermatomyositis/polymyositis. In this study, we demonstrate that NLR is also predictive in IPF, a disease in which inflammation is not thought to play a role, and indeed in which the use of immunosuppression in this disease has been shown to be harmful. It is unclear if NLR is alerting us to a potential role of inflammation in advancing interstitial inflammation or is highlighting a group of patients in which inflammation drives increased mortality from cardiovascular involvement. Disordered metabolism of carbohydrates, lipids, proteins, and hormones has been documented in lung, liver, and kidney fibrosis and metabolic dysregulation has been implicated in the pathogenesis of IPF, potentially offering a new target for fibrosis therapy.

The predictive ability of NLR may hint more directly at a role for neutrophilic inflammation in the pathogenesis of IPF. We have known for a long time that the percentage of neutrophils in the BAL of patients with IPF correlates with a poor outcome. Molyneaux et al. have shown that BAL neutrophilia is associated with both increased microbiome burden and progressive IPF, with subtle changes in the microbiome implicated in the initiation and progression of IPF in the absence of identified infection. The increased bacterial burden of IPF appears to be in the airway, proximal to the actual fibrotic remodelling of the parenchyma, with very low levels of bacteria identified in IPF parenchymal lung tissue. However, such changes are unlikely to cause increases in systemic neutrophilia and NLR in the absence of overt infection. In our study, we excluded patients diagnosed clinically with infection and started on antibiotics, and those in whom the C reactive protein (CRP) was greater than 20 mg/L.

If we do not think NLR is detecting occult infection, then why is it such a powerful marker? One developing line of enquiry is that the lung is responsible not just for gas-exchange but also plays a crucial role in leukocyte homeostasis. There is increasing evidence that the lung may orchestrate the disposal of aged neutrophils, by targeting them for recirculation to, and disposal in, the bone marrow. In a mouse model the inability of the lung to clear aged neutrophils resulted in a pulmonary fibrosis. As well as neutrophil activation, other groups have noted phenotypic changes in circulating leukocytes, for example CD28 downregulation on CD4 cells, perhaps reflecting T cell exhaustion, and 4 T cell genes (CD24, ICOS, LCK and ITK) are part of the 52 gene signature that is associated with a poor disease outcome. Interestingly we found that the neutrophil count is not as strong a predictor of mortality in IPF as NLR, suggesting that both neutrophil activation and lymphocyte exhaustion may be relevant.

Despite the reproducibility of our findings there are some caveats. We did not determine the specificity of NLR to IPF as opposed to other ILDs. However, we have previously reported that within the ILD cohort from RDE/NB/TS/UHL although high baseline NLR predict outcomes in IPF this was not the case in patients with chronic hypersensitivity pneumonitis. Secondly, most of the patients were in the pre-antibiotic era and many were treated with corticosteroids although we lack granular information on the doses and duration of such treatments. However, we have shown that neither the use of corticosteroids nor of antibiotics influenced either patient outcomes or the validity of NLR. Although surprising, this likely reflects: the small number of patients in these subgroups combined with the heterogeneity of our study populations when compared to clinical trial cohorts, making it underpowered to pick up the predicted favourable outcome with antibiotics or worse outcome with steroids. In addition, it is possible, although unproven, that these cohorts were exposed to lower doses (<20 mg) of prednisolone, compared to the doses of 0.5 mg/kg, average of 30 mg prednisolone, that were shown to be harmful in the PANTHER and other studies in which no excess adverse signal was seen once dose was reduced to 20 mg. The fact that despite this heterogeneity the prognostic potential of NLR still holds is encouraging. We have only limited longitudinal data, and there is a suggestion that patients will change their profiles but how this relates to their prognosis remains unclear. Nathan et al. found NLR change may be an even more robust prognostic biomarker than baseline NLR but may be less suitable as a predictive biomarker for patients receiving treatment with antibiotics. The main limitation of this retrospective study is linked to missing, and at times, poor quality data. In particular, we were lacking basic demographic data such as ethnicity, smoking status, and co-morbidities that were not consistently available across all cohorts. In addition, although all cases were incident IPF and CBC was measured at first appointment of IPF diagnosis, we did not consider time to diagnosis which has been shown to vary considerably in UK. However, our data does offer impetus to the idea that NLR should be evaluated as part of a prospective clinical trial as a secondary or an exploratory endpoint.
In summary, we have demonstrated and validated that NLR, an easily, widely available, cheap and reproducible test, is an independent prognostic biomarker that can be evaluated at diagnosis in patients with IPF and may inform future management of these patients. There is an enhanced cohort outcome prediction accuracy when NLR is added to GAP score suggesting that NLR may be useful not only as a stratification marker, but also a predictor for disease monitoring in IPF. One striking observation is that NLR correlates with lung function (FVC and TLC) and may be particularly helpful in assessing clinical priorities in situations where lung function is not easily available, such as in remote areas and during the pandemic, or cannot be performed by the patient. Further evaluation of the utility of NLR measurement for therapeutic decision making is warranted.

Contributors

Literature search and conceptualisation ICP and TAM. Figures TAM.

Study design TAM, JCP, CJJ. Data collection and curation: all authors. Data analysis: TAM, PG, JS, BG, CS, JP. Data interpretation: all authors. Writing – first draft: TAM and JCP. Review, editing and final approval: all authors.

Data sharing statement

Data collected for the study may be accessed after approval of a proposal and with a signed data access agreement with the individual investigators that manage the patient databases.

Declaration of interests

SLB reports consultancy fees from Boehringer Ingelheim (BII). PMG reports personal fees from BII and AstraZeneca (AZ) and Brainonix and lecturing honoraria from BII, Roche and Cipla. VK reports lecturing fees from Novartis, Roche, and BII. JCP reports consulting fees from Barratt therapies, AZ and lecturing honaria from The Limbic. EAR reports research grants from Novartis, Roche, and BI. Lecturing honoraria from BI, Roche and Cipla. Vineet reports consulting fees from Carrick therapeutics, AZ and lecturing honoraria from The Limbic. TAM reports research associate funding, NIHR. All other authors have nothing to disclose.

Acknowledgments

This work was supported by Breathin Matters, and undertaken at Exeter, Leicester, Imperial and UCLH/UCL BRCs who receive a proportion of funding from the Department of Health’s NIHR Biomedical Research Centres funding scheme. SL has research associate funding, NIHR. TAM held an independent studentship from GSK. TN held a joint studentship from the Cystic Fibrosis Trust and the British Lung Foundation (8319/06). JCP had an MRC New Investigator Award (MR/K004158/1). AD was supported in part by grant MR/N013794/1 for the GW4 BIOMED MRC DTP, awarded to the Universities of Bath, Bristol, Cardiff and Exeter from the Medical Research Council (MRC)/UKRI. CJJ was supported by an MRC grant (MR/V002538/1). RW was supported by an NIHR Academic Clinical Fellowship. BG was supported by awards from MRC (UK) the British Lung Foundation and the Alpha-1 Foundation.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2022.101758.

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Articles


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Update in diagnosis and management of interstitial lung disease

Authors: Theresia A Mikolasch, Helen S Garthwaite and Joanna C Porter

The field of interstitial lung disease (ILD) has undergone significant evolution in recent years, with an increasing incidence and more complex, ever expanding disease classification. In their most severe forms, these diseases lead to progressive loss of lung function, respiratory failure and eventually death. Despite notable advances, progress has been challenged by a poor understanding of pathological mechanisms and patient heterogeneity, including variable progression. The diagnostic pathway is thus being continually refined, with the introduction of tools such as transbronchial cryo lung biopsy and a move towards genetically aided, precision medicine. In this review, we focus on how to approach a patient with ILD and the diagnostic process.

KEYWORDS: Cryoscopic lung biopsy, idiopathic pulmonary fibrosis, interstitial lung disease

Introduction

Interstitial lung disease (ILD) is an umbrella term for over 200 different diseases that display considerable variation in terms of clinical course, treatment and prognosis. Broadly speaking, they can be subdivided into those with an identifiable cause and those without; the latter being referred to as idiopathic interstitial pneumonias. Clinical assessment aims to identify a possible cause; screening for features of systemic disease (e.g. connective tissue disease) or environmental triggers. Relevant exposures include pneumotoxic drugs, radiation therapy, occupational exposures (e.g. asbestos) or implicated allergens (hypersensitivity pneumonitis).

Distinguishing the various forms of pulmonary fibrosis is critical for determining correct management and for predicting prognosis; however, this is often obfuscated by the fact the lung has a limited repertoire in response to injury and, consequently, a finite number of disease patterns. In essence, all ILD is characterised by variable degrees of inflammation and fibrosis, not only between diseases, but also among individuals with the same disease (Fig 1). In inflammation dominant disease, the histology is that of organising pneumonia or non-specific interstitial pneumonitis, while in fibrosis dominant disease, one would expect to see usual interstitial pneumonitis (UIP) – characterized by fibroblastic foci and only mild to moderate inflammation. These histological patterns are associated with specific radiological features, the recognition of which may abrogate the need for a formal biopsy and tissue diagnosis.

Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is the most common of the idiopathic interstitial pneumonias, with an incidence of approximately 6,000 cases per year in the UK, affecting mainly older males. Median survival is worse than many cancers at just 3 years and the disease accounts for 5,300 deaths each year in the UK.1 IPF is a growing problem, with an annual increase in incidence of 11% between 1991 and 2003, a rise that is only partly explained by an ageing population.2

As already alluded to, distinguishing IPF from other ILDs, including other idiopathic forms, is important for multiple reasons. IPF does not respond to immunosuppressive therapy; in fact, immunomodulation may worsen outcomes.3 By contrast there is evidence, particularly in systemic sclerosis associated ILD,4 of benefit from cyclophosphamide and multiple case reports suggest a potential role for rituximab5 as salvage therapy in connective tissue disease-ILD. In addition, there are now two drugs, pirfenidone and nintedanib, approved by the National Institute for Health and Care Excellence (NICE) for IPF; however, at an annual cost of around £26,000 per patient and the potential for significant side effects, accurate disease identification is essential. Finally, IPF has a worse prognosis than other ILD; therefore, a definitive diagnosis allows for timely involvement of palliative care physicians and consideration of lung transplantation.

Pathogenesis of idiopathic pulmonary fibrosis

The pathogenesis of IPF is complex and poorly understood, but involves aberrant wound healing in the context of repetitive alveolar injury. This results in abnormal fibroblast proliferation, differentiation and activation, which in turn drives expansion of the extracellular matrix with loss of normal lung architecture. Inflammation plays a less dominant role. This pathogenesis is illustrated schematically in Fig 2.
Fig 2. The pathogenesis of idiopathic pulmonary fibrosis. 1 – In an initiating phase, there is lung alveolar epithelial damage with loss of the normal lung architecture and disruption of the basement membrane across which gas exchange takes place. With further epithelial damage and apoptosis, comes upregulation of epithelial integrins, such as $\alpha v\beta 6$, and a phase of fibroproliferative repair dominates – driven by high levels of TGF-$\beta$. Released in an inactive form, this cytokine requires an activation step facilitated by integrins that bind the Arg-Gly-Asp (arginine-glycine-aspartic acid; RGD) motif of pro-TGF$\beta$ and promote its cleavage and activation. 2 – Locally activated TGF-$\beta$ drives the recruitment of fibroblasts and a feed-forward cycle of further TGF-$\beta$ production. 3 – Under these conditions, fibroblasts differentiate into myofibroblasts that express high levels of integrin $\alpha v\beta 6$, are resistant to apoptosis and lay down a collagen matrix. 4 – Once collagen has been laid down in a lung, the architecture of which is already distorted, gas exchange is no longer efficient. There is a change in the vasculature of the lung parenchyma with both fall-out of blood vessels and neo-angiogenesis driven by local production of vascular endothelial and platelet derived growth factors (VEGF and PDGF). At this final phase, the lung is irreversibly scarred.

AEC = alveolar epithelial cell; PDGF = platelet-derived growth factor; TGF-$\beta$ = transforming growth factor beta; TNF-$\alpha$ = tumour necrosis factor alpha; VEGF = vascular endothelial growth factor.

Fig 1. Schematic classification of interstitial lung diseases according to aetiology. The finding of histological usual interstitial pneumonitis in a patient with an idiopathic interstitial pneumonia leads to the specific diagnosis of idiopathic pulmonary fibrosis. NSIP = non-specific interstitial pneumonitis.
Genetics in diagnosis and management of idiopathic pulmonary fibrosis

Although the initiating events in IPF are poorly understood, the disease is likely to be the result of environment exposures in genetically susceptible individuals. Certainly, it is estimated that approximately 20% of idiopathic interstitial pneumonias have a genetic component and familial cases (referred to as familial interstitial pneumonias) were first described in the 1950s. The majority of these familial interstitial pneumonias are autosomal dominant with incomplete penetrance, but some may arise de novo. The most commonly affected genes are those involved in surfactant processing and telomere biology. At present, routine genetic testing is not recommended; however, ILD patients with at least one affected first degree relative should be offered the opportunity to enrol in the UK-wide 100,000 Genomes Project, whereby they undergo whole genome sequencing.

Genetics also have a proven role in sporadic IPF. Polymorphisms in the promoter for the gene encoding the salivary mucin, 5b (MUC5B) and for the Toll-interacting protein (TOLLIP) are both associated with an increased risk of developing IPF, although both result in a relatively mild phenotype. These genetic variants provide the first possible genetic susceptibility factors for IPF (Table 1), precipitating immunoglobulins against organic antigens and serum angiotensin-converting enzyme. These tests alone rarely confirm the diagnosis and there is potential for both false positive (particularly autoantibodies in older patients) and false negative (failure to identify an antigen and IgG does not exclude hypersensitivity pneumonitis) results; however, they can be useful in helping direct further diagnostics.

Pulmonary function tests are key in appraising these patients and while they rarely refine the specific diagnosis in individuals with proven ILD, they inform on disease severity at baseline and response to treatment over follow up.

Radiological work up

Chest radiographs

A chest X-ray is often the first radiological investigation in ILD patients and while it is rarely sufficient to make a confident diagnosis, X-ray can play a role in establishing disease chronicity and progression.

High-resolution computerised tomography

High-resolution computerised tomography (HRCT) of the thorax has revolutionised the diagnosis and classification of ILD and, in many cases, removes the need for invasive diagnostic procedures. However, the quality of the images is dependent on the scanning protocol employed (Box 1).

The American Thoracic Society/European Respiratory Society (ATS/ERS) 2011 consensus statement provides criteria for a definitive UIP pattern on HRCT (Fig 3), with the presence of these conferring a sensitivity of approximately 40%, but a specificity of 95% for histological UIP. The main discriminating feature for UIP is the presence of honeycombing; however, typical CT appearances are only present in two thirds of patients and it is in the remaining third of cases that biopsy may have a role.

Table 1. Autoantibodies in connective tissue ILDs

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Associated connective tissue disease</th>
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<tbody>
<tr>
<td>ANA (&gt;1:320)</td>
<td>Many</td>
</tr>
<tr>
<td>RF (&gt;60 IU/mL)</td>
<td>RA, Sjögren’s syndrome, SLE</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>RA</td>
</tr>
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<td>Anti-centromere</td>
<td>Systemic sclerosis</td>
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<tr>
<td>Anti-nuclear ANA</td>
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<tr>
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<td>SLE</td>
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<td>Anti tRNA synthetase</td>
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</tr>
<tr>
<td>Anti-U3 RNP</td>
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</tr>
<tr>
<td>ANCA panel</td>
<td>Vasculitides</td>
</tr>
<tr>
<td>Anti-tRNA synthetase (Scl-70)</td>
<td>Systemic sclerosis</td>
</tr>
</tbody>
</table>

| ANA = antinuclear antibody; ANCA = Anti-neutrophil cytoplasmic antibodies; Anti-CCP = anti-cyclic citrullinated peptide antibody; anti-dsDNA = anti-double stranded DNA; anti-FM-Scl = anti-polymyositis-scleroderma; anti-RNP = antinuclear protein; ILD = interstitial lung disease; MCTD = mixed connective tissue disease; RA = rheumatoid arthritis; RF = Rheumatoid factor; SLE = systemic lupus erythematosus. |

Box 1. The ATS/ERS consensus statement for the diagnosis of IPF set out criteria for the optimal HRCT technique for evaluation of ILD

Optimal HRCT technique for evaluation of ILD

- Non-contrast scans obtained on full inspiration without respiratory motion.
- Contiguous or non-contiguous axial scans with thin sections, reconstructed at ≤2 cm intervals.
- Reconstructed slice collimation ≤2 mm.
- High resolution reconstruction algorithm.
- Field of view to include lungs only.
- Expiratory scans are helpful to exclude lobular air trapping suggestive of hypersensitivity pneumonitis.
- Prone scans if dependent density obscures detail on supine images.
- Optional coronal and sagittal reconstructions if volumetric images are obtained.

ATS = American Thoracic Society; ERS = European Respiratory Society; HRCT = high resolution computerised tomography; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis.

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Bronchoscopic biopsy (TBB) with standard forceps is a safe, minimally invasive technique, but does not always provide adequate lung tissue to establish a final diagnosis. The biopsies are subject to crush artefact and may not be representative in all six cases, further investigations led to a final diagnosis of chronic hypersensitivity pneumonitis.

Transbronchial cryo lung biopsy (TBCLB) was first described in 2008.\textsuperscript{11} It has since been shown to be a safe, minimally invasive and effective diagnostic tool for the histological diagnosis of ILD, with a diagnostic yield of up to 74–80\%.\textsuperscript{11–15} The advantage of TBCLB over TBB lies in the larger specimen size, with a mean size of 9–64 mm\textsuperscript{2}.\textsuperscript{11–20} In addition, the technique avoids crush or bleeding artefact, which can distort the tissue architecture (Fig 4). The published data on TBCLB shows a safety profile that is comparable to TBB, with bleeding post biopsy in around 10% of cases, all of which was controlled bronchoscopically. The mean rate of pneumothorax requiring chest drain insertion is around 4%, although there is a wide variation between centres. Exacerbations of ILD are rare (0.5%) and only one mortality has been reported (0.2%).\textsuperscript{11–20}

Pajares et al\textsuperscript{2} conducted a prospective randomised trial comparing TBCLB with TBB and demonstrated a mean specimen size for TBCLB samples of 14.7 mm\textsuperscript{2} (versus 3.3 mm\textsuperscript{2} for TBB biopsy, p<0.001), resulting in a histological diagnosis in 74.4% of patients versus 34.2% in the TBB group (p<0.001). When comparing this technique with surgical lung biopsy there are various potential advantages. General anaesthesia is not necessary and the procedure can be performed as a day case with uncomplicated cases returning home the same day. Future clinical trials and an increase in real world experience of TBCLB is likely to cement its use for selected cases, potentially reducing the number of surgical lung biopsies performed.

**Surgical lung biopsy**

Surgical lung biopsies are the current gold standard for obtaining histological material in the diagnosis of clinically and radiologically unclassifiable ILD. It is usually performed via the less invasive video-assisted thoracoscopic surgical (VATS) approach. As previously described, using the criteria set out in the 2011 ATS/ERS consensus statement, about two thirds of IPF cases can be diagnosed on the basis of typical clinical and radiological findings of UIP (Fig 5); however, only 7.5–12% of suspected IPF patients undergo surgical lung biopsy in the UK.\textsuperscript{21} This reflects clinicians’ reluctance to refer patients for a procedure associated with a significant mortality and morbidity. The average hospital stay associated with VATS biopsy is 2–4 days,\textsuperscript{22} with mortality rates of 3–4% and an overall complication rate of up to 16%.\textsuperscript{23} Common complications include persistent air leak, exacerbations of underlying ILD due to mechanical stress of single lung ventilation, bleeding and delayed wound healing. In addition, 57% of patients report pain
at the incision site 6–12 months after surgery. It is also worth remembering that surgical lung biopsy does not guarantee a definite pathological diagnosis, with diagnosis rates ranging from 34–100%.22,23

Multidisciplinary team

Taking into consideration the various investigations involved in ILD diagnosis it is clear that no single diagnostic test can provide a confident answer. A consensus approach by a multidisciplinary team (MDT) with expertise in ILD is thus considered the gold standard (Fig 6). The MDT integrates all available data at several stages of the work up. This not only improves inter-observer agreement and diagnostic confidence, but may also prevent unnecessary surgical biopsies, while identifying patients in whom a biopsy may effectively contribute to the diagnosis.24 Current NICE guidelines recommend that IPF should only be diagnosed by MDT consensus and stipulates a minimum MDT composition.26

Novel therapies in idiopathic pulmonary fibrosis

There has been a dramatic increase in clinical trial activity in IPF in recent years, with the discovery and approval of two new anti-fibrotic drugs (pirfenidone and nintedanib) heralding a new era in the disease. While these novel anti-fibrotic agents have been shown to slow the decline in forced vital capacity (FVC), they neither halt progression nor reverse existing fibrosis. In part because of considerable cost, their use is restricted by NICE to patients fulfilling certain criteria – namely a predicted FVC of 50–80%, thereby excluding patients at each extreme of the disease process and those with spuriously maintained FVC due to concurrent emphysema. Given these restrictions, as well as the limitations of these therapies, the importance of non-pharmacological therapy, such as pulmonary rehabilitation, plus the enrolment of patients into clinical trials (Fig 7) should not be underestimated.

Novel biomarkers

The need to distinguish the different idiopathic interstitial pneumonias has driven the search for novel diagnostic biomarkers. In addition, there are marked survival differences even within specific groups, such as IPF. Biomarkers that can identify these phenotypes are needed for clinical decision making, but they also have the potential to aid cohort enrichment in clinical trials. Previous landmark studies have beautifully illustrated this need, with variable rates of decline in placebo arms leading to inconsistent results and a delay in drugs being approved.25
Fig 6. The role of specialist multidisciplinary teams and specialist referral centres in the diagnosis and management of interstitial lung disease.
CTD = connective tissue disease; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; LAM = lymphangioleiomyomatosis; LCH = Langerhans cell histiocytosis; MDT = multidisciplinary team; NSIP = non-specific interstitial pneumonitis.

Fig 7. Schematic interstitial lung disease treatment algorithm. *No robust evidence for managing exacerbations with variation between centres, should be discussed with specialist centre if possible. BAL = bronchoalveolar lavage; GOR = gastro-oesophageal reflux; HRCT = high-resolution computerised tomography; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; MDT = multidisciplinary team; PFT = pulmonary function test; PHT = pulmonary hypertension.
To date, efforts have focused on serum biomarkers that are relatively easy to access and novel imaging modalities that potentially inform on disease activity within the lung. In particular, positron emission tomography (PET) allows non-invasive measurement of cellular metabolism in vivo. The $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) PET signal is consistently raised in ILD and is both stable and reproducible (Fig 8). In a population of over 200 ILD patients, we have shown that baseline measures of $^{18}$F-FDG uptake on PET are related to patient survival in both IPF and other idiopathic interstitial pneumonias (unpublished data). High pulmonary $^{18}$F-FDG uptake is associated with poor survival, giving additional information to pulmonary function testing and, thus, could become a valuable part of the initial work up in newly diagnosed patients.

Conclusion

The diagnosis of ILD is a challenging and involved process. It relies on detailed history taking and the integration of various investigations and specialties. The relative rarity of these diseases makes distinguishing subtypes even more difficult for clinicians with a mixed respiratory case workload and, thus, infrequent exposure to ILD. Having said this, the incidence of ILD is increasing and there is potential for specialist centres to become overwhelmed with patients, putting a greater than ever emphasis on collaboration with referring centres and a concerted effort to employ a hub and spoke model.

This has the added advantage of facilitating a more patient-centred approach, minimising the need for unnecessary travel and facilitating access to ancillary local services, such as pulmonary rehab, oxygen providers and palliative care services.

Irrespective of expertise, uncertainty is inherent in the diagnosis of these diseases, although arguably encountering ILD on a frequent basis allows the physician to become more comfortable with these uncertainties, thus embracing the concept of continuous diagnostic review. The hope remains that, in time, reliable, non-invasive biomarkers will identify disease subtypes, predict prognosis and potentially replace the need for biopsy. Much of the heterogeneity seen in IPF may be explained by the existence of endotypes, in other words, mechanistically different disease subtypes, which consequently exhibit very different responses to therapy. Therefore, future treatments have the potential to be greatly influenced by identifying these groups through the use of genetic testing and a move towards personalised disease management.

Conflicts of interest

The authors have no conflicts of interest to declare.

Note

This article was originally published in the 2016 Clinical Medicine supplement Horizons in Medicine 28. All articles in this supplement are available online at www.clinmed.rcjournal.org/content/16/Suppl_6

References

Corrigendum: Headache in an HIV positive patient: diagnostic challenges and approach to treatment

Authors: Andrew Creamer, A Stefanos Ioannidis, B Thomas Wilhelm, C Tabitha Mahungu D and Marc Lipman E

Tabitha Mahungu’s name was published with the incorrect spelling. The correct spelling is printed above.
EDITORIAL

Transbronchial cryobiopsy in the diagnosis of interstitial lung disease: A cool new approach
APPENDIX 2- INHALE PROTOCOL
**TITLE PAGE**

**Division:** Worldwide Development

**Information Type:** Clinical Protocol

<table>
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<th>Study to assess inhaled drug distribution in the distal lung and interstitium using cryobiopsy samples from subjects with suspected Interstitial Lung Disease undergoing cryobiopsy for clinical reasons</th>
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**Compound Number:** Non-compound specific

**Development Phase** | NA

**Effective Date:** 21-SEP-2016

**Subject:** Cryobiopsy, Lung biopsy, Interstitial Lung Diseases (ILD), Inhaled drug distribution/deposition.

**Author(s):** Jordan, Faron; Oballa, Eunice; Fahy, William A; Birbeck, Emma; Morrell, Josie; Marshall, Peter; Jarvis, Emily; Vahdati-Bolouri, Mitra

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21 September 2016
**MEDICAL MONITOR/SPONSOR INFORMATION PAGE**

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<tr>
<th>Role</th>
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<th>After-hours Phone Number</th>
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<td>[Redacted]</td>
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Regulatory Agency Identifying Number(s): NA
INVESTIGATOR PROTOCOL AGREEMENT PAGE

I confirm agreement to conduct the study in compliance with the protocol.
I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described study.
I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.
Investigator Name: ________________________________

________________________________________________________________________
Investigator Signature Date
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# ABBREVIATIONS AND TRADEMARKS

## Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>BTS</td>
<td>British Thoracic Society</td>
</tr>
<tr>
<td>CONSORT</td>
<td>Consolidated Standards of Reporting Trials</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>DRE</td>
<td>Disease Related Events</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>eCRF</td>
<td>Electronic Case Report Form</td>
</tr>
<tr>
<td>FEV1</td>
<td>Forced Expiratory Volume in 1 second</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>GSK</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>HRCT</td>
<td>High Resolution Computed Tomography</td>
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<tr>
<td>ICH</td>
<td>International Conference on Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use</td>
</tr>
<tr>
<td>IEC</td>
<td>Independent Ethics Committee</td>
</tr>
<tr>
<td>IIP</td>
<td>Idiopathic Interstitial Pneumonia</td>
</tr>
<tr>
<td>ILD</td>
<td>Interstitial Lung Disease</td>
</tr>
<tr>
<td>IPF</td>
<td>Idiopathic Pulmonary Fibrosis</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review board</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>MALDI-MS</td>
<td>Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry</td>
</tr>
<tr>
<td>mcg</td>
<td>micrograms</td>
</tr>
<tr>
<td>MCH</td>
<td>Mean Corpuscular Haemoglobin</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean Corpuscular Volume</td>
</tr>
<tr>
<td>MedDRA</td>
<td>Medical Dictionary for Regulatory Activities</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetres</td>
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<tr>
<td>MSI</td>
<td>Mass Spectrometry Imaging</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
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<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
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<tr>
<td>SGPT</td>
<td>Serum glutamic-pyruvase transaminase</td>
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<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>SPC</td>
<td>Summary of Product Characteristics</td>
</tr>
<tr>
<td>SRM</td>
<td>Study Reference Manual</td>
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<tr>
<td>TBCB</td>
<td>Trans Bronchial Cryo Biopsy</td>
</tr>
<tr>
<td>UCL</td>
<td>University College London</td>
</tr>
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<td>UCLH</td>
<td>University College London Hospitals</td>
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</table>
UK | United Kingdom
---|---
VATS | Video-Assisted Thorascopic Surgical procedure

**Trademark Information**

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1. PROTOCOL SYNOPSIS FOR STUDY 205053

Rationale

This study will utilise samples obtained by transbronchial cryobiopsy (TBCB) bronchoscopic technique to assess the distribution of inhaled drugs in the lungs of patients with fibrotic lung disease using mass spectrometric techniques.

The assessment of drug distribution to the relevant lung compartments is essential when developing new inhaled therapies. Interstitial lung disease (ILD) is often characterised by pathology in the distal lung and the ability to deliver inhaled medicines to these areas is poorly understood. Following inhalation of ipratropium bromide, TBCB provides a minimally invasive, safe alternative compared to Video-Assisted Thorascopic Surgery (VATS) for providing tissues from distal lung of patients with fibrotic lung disease to assess deposition using mass spectrometric techniques. This study will recruit patients with suspected ILD who are referred for TBCB at University College London Hospitals (UCLH) as part of their diagnostic testing with a maximum of two additional TBCB samples taken for this study if safe to do so. One to three endobronchial forceps biopsy samples will be taken from up to 5 patients to allow comparison of proximal and distal drug distribution.

Objective(s)/Endpoint(s)

<table>
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<th>Endpoints</th>
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<tr>
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<tr>
<td>• To evaluate the ability of inhaled ipratropium bromide to reach the distal lung, by analysing transbronchial cryobiopsies from fibrotic regions of the lung</td>
<td>• Images and data generated using mass spectrometric techniques and histology showing the distribution of ipratropium bromide within the cryobiopsy samples taken from suspected ILD patients.</td>
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<tr>
<td>Secondary</td>
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<tr>
<td>• To assess ipratropium bromide distribution in endobronchial forcep biopsy samples</td>
<td>• Image and data generated using mass spectrometric techniques and histology showing the distribution of ipratropium bromide within the endobronchial samples taken from suspected ILD patients</td>
</tr>
<tr>
<td>• To compare the distribution of inhaled ipratropium bromide in proximal and distal lung</td>
<td>• Mass spectrometry and histology data showing distribution of inhaled ipratropium bromide in the proximal and distal lung.</td>
</tr>
<tr>
<td>Objectives</td>
<td>Endpoints</td>
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<tr>
<td>-----------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
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<tr>
<td>• Development of other mass spectrometry/experimental techniques for sample analysis e.g. for metabolomics, determination of collagen subtypes, drug quantitation.</td>
<td>• Mass spectrometry/experimental data identifying collagen subtypes, metabolomics and ipratropium bromide concentrations in biopsy samples.</td>
</tr>
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</table>

**Overall Design**

This is a single centre feasibility study to evaluate the ability of inhaled ipratropium bromide to reach the distal lung by processing TBCB and endobronchial forcep biopsy samples using mass spectrometry including but not limited to Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS) and histology techniques.

The study is non-interventional and the subjects will be undergoing TBCB for clinical reasons as part of their diagnostic testing.

**Treatment Arms and Duration**

All patients who consent to the study through an ethically approved informed consent process will receive nebulised ipratropium bromide before the cryobiopsy procedure. Up to 2 additional biopsy samples will be taken for this study after samples required for diagnosis have been taken. In addition, one to three endobronchial forceps biopsy samples will be taken from up to 5 patients to allow comparison of proximal and distal drug distribution.

**Type and Number of Subjects**

Up to 20 individuals over the age of 18 years (inclusive) with suspected ILD, referred for TBCB for clinical reasons, as part of their diagnostic work up, will be recruited to the study.

**Analysis**

Data generated from this study will be semi-quantitative- MALDI-MS imaging and histology therefore no formal statistical analysis will be taken. No formal hypothesis will be tested.
2. INTRODUCTION

2.1. Study Rationale

The assessment of drug distribution in the relevant lung compartments is essential when developing new inhaled therapies targeting the peripheral lung. Radionuclide imaging is well established for quantifying the whole lung deposition of inhaled drugs, but the assessment of regional lung deposition is less straightforward, because of the complex nature of the lung anatomy [Newman, 2011]. Direct assessment of the distribution of drugs in different compartments of the lung would allow for a more complete understanding of the local deposition and pharmacological action. However, this has been challenging due to the invasive nature of conventional sampling techniques of the distal lung.

Until very recently, the main approach to obtaining good quality lung tissue samples was a surgical lung biopsy, usually performed via the less invasive Video-Assisted Thorascopic Surgical (VATS) approach rather than an open thoracotomy biopsy. VATS biopsies in patients with interstitial lung diseases have fallen out of favour over the last few years due to an improvement in radiological diagnosis and also a reluctance of clinicians to refer patients for a procedure associated with significant mortality and morbidity. The average hospital stay associated with VATS biopsy is 2 to 4 days [Morris, 2014]. Mortality has been reported as 3 to 4% and overall complication rate up to 16% [Kaarteenaho, 2013]. 57% of patients report pain at the incision site 6 to 12 months after surgery [Maguire, 2006]. Other common complications include: persistent air-leak; exacerbation of underlying interstitial lung disease (ILD) due to mechanical stress of single lung ventilation; bleeding and delayed wound healing.

This has had a knock-on effect on tissue availability for basic science research as well as drug development in the field of pulmonary fibrosis.

The advent of transbronchial cryobiopsy (TBCB) provides a minimally invasive, safer alternative to VATS lung biopsy and therefore increases the availability of tissue from the lung periphery to study the ability of inhaled drugs to reach the distal lung of patients with fibrotic lung disease.

This study will utilise samples obtained by this novel, bronchoscopic technique to assess the distribution of inhaled drugs in the lungs of patients with fibrotic lung disease using mass spectrometric techniques. The Matrix assisted laser desorption/ionization-Mass Spectrometry (MALDI MS) rationale is based on previous work published on inhaled ipratropium bromide deposition [Fehniger, 2011] and supporting work carried out in a rat model.

Knowledge of drug distribution in the lung interstitium could facilitate inhaled drug development and therefore directly benefit patients by evaluating the ability of a potential drug to reach its target tissue and its local molecular effects.
2.2. Brief Background

ILD is a term used to collectively describe a group of diffuse parenchymal lung disorders that are diagnosed depending on clinical, histopathological and radiological presentation [Castelino, 2010]. When there is no clear cause to the development of an ILD, then a diagnosis of Idiopathic Interstitial Pneumonia (IIP) is made, the most common of which being Idiopathic Pulmonary Fibrosis (IPF). IPF is a progressive, life threatening disease that is responsible for 5000 deaths every year in the United Kingdom (UK), with the median patient survival after diagnosis ranging from 2.5 to 3.5 years [American Thoracic Society, 2000]. In addition, studies have shown the incidence of IPF continues to rise in the UK and USA while the survival time from diagnosis remains unchanged [Maher, 2013]. Respiratory diseases change the architecture of the lung and hence alter deposition and distribution of drugs following inhalation. It is therefore important to develop techniques to directly measure drug distribution within the lung.

Understanding of drug deposition in the lungs is of great value in improving efficiency in drug delivery of inhaled therapeutics. The current pharmacokinetic and scintigraphic methods used to assess deposition both have limitations, such as providing indirect measurements or requiring modification to the formulation of the study drug. Historically, direct measurement of drug concentrations at local sites in the lung has been difficult due to complications arising from sampling lung tissue from patients, as well as having the analytical techniques needed to assess drug concentrations in the tissue samples. This study will use the minimally invasive trans-bronchial cryobiopsy procedure to sample lung tissue from patients with interstitial lung disease. Mass spectrometry and histology techniques will be used to generate non-quantitative images of drug distribution within the biopsy samples.

2.2.1. Trans bronchial CryoBiopsy

An initial High Resolution Computed Tomography (HRCT) scan determines the location of biopsy. During bronchoscopy, patients are sedated, intubated and oxygen is insufflated through the nasal cannula continuously. A flexible bronchoscope is then passed into the lungs, under fluoroscopic guidance, to the target location, and lung biopsies are taken, in which 2 to 3 are used for diagnosis and the remainder are available for research. Known potential complications include bleeding, which is usually mild to moderate with no recorded cases of severe bleeding, as well as iatrogenic pneumothorax, with one case series reporting a rate of 20% [Ravaglia, 2016]. This study will utilise the development of the TBCB technique to obtain lung interstitium samples following nebulised delivery of ipratropium bromide, for analysis by, but not limited to, mass spectrometry and histology.

2.2.2. Endobronchial biopsy

Endobronchial samples will be taken from up to 5 subjects to allow comparison of proximal and distal drug distribution. The procedure will follow the same process as described in Section 2.2.1 however; biopsy forceps will be passed through the endoscopes central channel to allow sampling from the proximal region of the lung. Known complications are rare in the absence of a transbronchial biopsy and figures of
0.45% for minor bleeding and <0.5% for pneumothorax are quoted. (Du Rand, et al. Thorax 2013)

The samples will be analysed using the same techniques as used for the TBCB samples.

2.2.3. Pre-clinical/clinical supporting data

A study by [Fehniger, 2011] successfully demonstrated the utility of MALDI-MS to determine the distribution of ipratropium bromide in the lungs of patients with suspected airway obstruction or tumours. Ipratropium bromide was delivered via nebulisation for 10 minutes prior to bronchoscopy. Biopsy samples were taken by pinchers and prepared for Matrix Assisted Laser Desorption/Ionization- Mass Spectrometry Imaging (MALDI-MSI). Histology staining was performed on tissue slices and co-registered with ipratropium ion signal generated following mass spectrometry. Study rationale was also supported by pre-clinical support work at GlaxoSmithKline (GSK); the study utilised a scaled dose equivalent to the human clinical dose of ipratropium bromide 500 mcg and demonstrated adequate sensitivity and resolution for detection by MALDI-MSI. The compound was dosed in a nebulised formulation to rats for 5 minutes. 5 mm ex-vivo lung biopsy samples were taken at varying time points up to 65 minutes and were embedded into material suitable for MSI. MSI data confirmed detection of ipratropium throughout the time points, including at 65 minutes post nebulisation, suggesting that detection in human samples could be feasible even up to an hour post dose.

In addition, ipratropium bromide was found to be a suitable compound to use due to its good sensitivity for detection by mass spectrometric techniques such as MALDI.

3. OBJECTIVE(S) AND ENDPOINT(S)

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td></td>
</tr>
<tr>
<td>• To evaluate the ability of inhaled ipratropium bromide to reach the distal lung, by analysing transbronchial cryobiopsies from fibrotic regions of the lung</td>
<td>• Images and data generated using mass spectrometric techniques and histology showing the distribution of ipratropium bromide within the cryobiopsy samples taken from suspected ILD patients.</td>
</tr>
<tr>
<td><strong>Secondary</strong></td>
<td></td>
</tr>
<tr>
<td>• To assess ipratropium bromide distribution in endobronchial forcep biopsy samples</td>
<td>• Image and data generated using mass spectrometric techniques and histology showing the distribution of ipratropium bromide within the endobronchial samples taken from suspected ILD patients.</td>
</tr>
<tr>
<td>Objectives</td>
<td>Endpoints</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>• To compare the distribution of inhaled ipratropium bromide in proximal and distal lung</td>
<td>• Mass spectrometry and histology data showing distribution of inhaled ipratropium bromide in the proximal and distal lung.</td>
</tr>
</tbody>
</table>

**Exploratory**

• Development of other mass spectrometry/experimental techniques for sample analysis e.g. for metabolomics, determination of collagen subtypes, drug quantitation.  
  • Mass spectrometry/experimental data identifying collagen subtypes, metabolomics and ipratropium bromide concentrations in biopsy samples.

### 4. STUDY DESIGN

#### 4.1. Overall Design

This is a single centre feasibility study to evaluate the ability of nebulised ipratropium bromide to reach the distal lung by analysing TBCB samples using mass spectrometric and histology techniques. In addition, endobronchial forceps biopsy samples taken from up to 5 patients and analysed using mass spectrometric and histology techniques will allow comparison of proximal and distal drug distribution.

The study is not a clinical trial of a medicinal product.

Subjects will be required to participate in the following:

**Screening:**

This study will recruit patients with suspected ILD who are referred for TBCB at University College London Hospitals (UCLH) as part of their diagnostic tests. Patients will be seen by an ILD specialist and a bronchoscopist who will perform the biopsy procedure. The subjects will be screened after the informed consent document has been signed up to 6 weeks prior to the biopsy procedure. Safety assessments will be carried out as described in the time and events table (Section 7.1) in order to assess eligibility for the procedure and inclusion in this study.

**Biopsy Procedure:**

Patients will be admitted to the endoscopy unit before the procedure. Following additional safety checks as shown in the time and events table (Section 7.1), the subjects will receive nebulised ipratropium bromide 500 mcg for 10 minutes immediately before undergoing bronchoscopy. The patients will be sedated for the procedure. Cryobiopsy samples required for routine clinical diagnosis will be taken first; 1 or 2 samples for this study will be taken after and only if, in the professional judgement of the bronchoscopist
taking additional samples, it will not impact on the safety of the patient. One to three endobronchial forceps biopsy samples will be taken from up to 5 patients to allow comparison of proximal and distal drug distribution. Research samples will be embedded in a suitable material and frozen for analysis as detailed in the Study Reference Manual (SRM).

Follow up:

Seven to fourteen days after the procedure UCLH will make a phone call to the patient.

Details of patient medical history, clinical examination and investigation results will be extracted from patient records. This data will then be entered onto the electronic case report form (eCRF).

4.2. Study Duration

The study will start when the first patient consents to the study and undergoes cryobiopsy. Up to 20 patients will be recruited. In stream analysis will be performed; study recruitment may be stopped if the results are clear and further data is unlikely to change the outcome of the study.

The study will have a single time point visit; subjects recruited will already be undergoing the cryobiopsy procedure as part of their routine clinical diagnosis. Study specific follow-up will occur 7 to 14 days after the procedure.

Subjects will be contacted by their referring clinician with the results of the biopsy.

4.3. Type and Number of Subjects

Up to 20 subjects over the age of 18 years (inclusive) presenting to the ILD services at UCLH with suspected fibrotic ILD, who require a TBCB as part of their diagnostic work up, will be enrolled.

4.4. Design Justification

The ILD Unit at UCLH is currently the only unit in the UK offering bronchoscopic, TBCB as part of the diagnostic pathway of patients with fibrotic ILD. Therefore, study subjects will not be requested to undergo TBCB specifically for research purposes.

The additional time added to the routine procedure by administering ipratropium bromide and obtaining a research biopsy will be approximately 15 minutes.

Taking both TBCB and endobronchial forceps samples will allow the assessment of drug deposition in the proximal and distal lung.
The use of cryobiopsy samples to assess the deposition of inhaled drugs (for example ipratropium bromide) using mass spectrometric techniques can determine drug distribution in the lungs of patients with fibrotic lung disease. The use of MALDI-MS techniques to image drug distribution in tissue biopsy samples has been successfully demonstrated in patients with suspected tumours or obstruction caused by Chronic Obstructive Pulmonary Disease (COPD) [Fehniger, 2011].

This study, to our knowledge is the first to investigate drug distribution in the lungs of patients with ILDs. Data from this study could be used to facilitate drug development programs by potentially shortening drug development time, as it not only evaluates a compound’s ability to reach its target tissue, but also has the potential to evaluate its local molecular effects.

4.5. Dose Justification

A clinically approved and widely used dose of nebulised ipratropium bromide (500 mcg) will be used to allow analysis of how inhaled drugs are distributed in the lung interstitium of patients with fibrotic lung disease.

The pre-clinical support study in rats also demonstrated that a single dose of ipratropium bromide, equivalent to 500 mcg human dose level, had sufficient sensitivity and specificity for MALDI-MS imaging.

4.6. Benefit: Risk Assessment

Subjects will be informed during consent that there will be no intended clinical benefit to the subjects personally from taking part in the study. It may be that research carried out on the samples provided will help future patients.

Bronchoscopy patients will already be having a biopsy as part of their diagnosis procedure. The risks associated with TBCB will be explained to the patient as part of their routine, clinical consent procedure. The risk of pneumothorax in the patient cohort at UCLH is approximately 15% and the risk of moderate bleeding around 10%. The risks of an additional biopsy taken for this study is difficult to estimate but will not significantly differ from the risks discussed with the patient during consenting.

Ipratropium bromide is a clinically approved and widely used bronchodilator drug with a well known safety profile.
### Risk of bronchoscopy-associated pneumothorax

<table>
<thead>
<tr>
<th>Study Procedures</th>
<th>Mitigation Strategy</th>
<th>Summary of Data &amp; Rationale for Risk</th>
<th>Potential Risk of Clinical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.6.1. Risk Assessment and Management</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CONFIDENTIAL</td>
</tr>
<tr>
<td>Study Procedures</td>
<td>Mitigation Strategy</td>
<td>Summary of Data/Reason for Risk</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>---------------------</td>
<td>--------------------------------</td>
<td></td>
</tr>
<tr>
<td>Bronchoscopy</td>
<td>Medical staff, performed and monitored by trained bronchoscopists and cytopathologists will be expelled and diagnosed</td>
<td>Potential risk of clinical significance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypoxia (Oxygen saturation &lt;88%) is expected, as per the local protocol, will be provided until oxygen saturation &gt;88%. Supplemental oxygen bronchoscopy. If oxygen levels drop to 88%, oxygen saturation may drop during procedure.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procedure as per routine clinical practice</td>
<td>Possible cardiac and respiratory complications following propofol infusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study patients will be monitored for any signs of complications throughout the procedure</td>
<td>Protocol infusion will be administered by a qualified anaesthetist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study patients will be monitored for any signs of complications throughout the procedure</td>
<td>Patients will receive propofol infusion during bronchoscopy as per routine clinical practice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue samples will be obtained for clinical research</td>
<td>Unlikely to increase the risk: additional research sample(s) taken is unlikely to increase the risk.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchoscopy, biopsy of tissue samples required for clinical research</td>
<td>Hypoxia (Oxygen saturation &lt;88%) is expected, as per the local protocol, will be provided until oxygen saturation &gt;88%. Supplemental oxygen bronchoscopy. If oxygen levels drop to 88%, oxygen saturation may drop during procedure.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential Risk of Clinical Significance</td>
<td>Summary of Data/Rationale for Risk</td>
<td>Mitigation Strategy</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Study Procedures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
| **Bleeding**                           | Subjects will be having bronchoscopy as part of their diagnosis procedure. Bleeding is a risk associated with the procedure regardless of study participation. | The subjects will be assessed continuously throughout the procedure as per routine practice for patients undergoing TBCB. The following prophylactic interventions may be used as required;  
  - Prophylactic intravenous (IV) tranexamic acid  
  - Prophylactic endobronchial adrenaline  
  - Prophylactic placement of Fogarty balloon as an endobronchial blocker |
|                                        |                                   |                     |
| **Other**                              |                                   |                     |
| Ipratropium bromide side effects       | Potential for hypersensitivity reaction such as bronchospasm but this is very rare:  
  (>1/10,000, <1/1000)  
  Side effects include a dry mouth, skin flushing, nausea, palpitations and headache | Patients with known hypersensitivity to atropine or ipratropium bromide will be excluded from the study  
  Continuous clinical monitoring as per standard practice for patients undergoing TBCB.  
  In case of bronchospasm, bronchodilators such as salbutamol are readily available. |
4.7. **Benefit Assessment**

There will be no intended clinical benefit to the subjects personally from taking part in the study. However, participation in this study will be contributing to the process of developing new therapies in an area of unmet need; research carried out on the samples provided may help future ILD patients. Patients recruited in to this study will continue to have their regular medical treatment as per routine practice.

4.8. **Overall Benefit: Risk Conclusion**

Subjects recruited to this study will already be having TBCB for diagnostic purposes; up to 2 additional TBCB samples from all subjects and up to 3 endobronchial samples in a maximum of 5 subjects will be collected for research. A single dose of ipratropium bromide will be dosed before having the biopsy procedure. Ipratropium bromide is a clinically approved and widely used bronchodilator drug with a well known safety profile. Taking into account the measures taken to minimize risk to subjects participating in this study, the potential risks identified in association with having the additional biopsy samples are justified by the anticipated benefits that may be afforded to subjects with ILDs.

5. **SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA**

Deviations from inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or patient safety. Therefore, adherence to the criteria as specified in the protocol is essential.

Patients may be withdrawn from the study at their own request. Patients will be made aware that this will not affect their future care. Patients will also be made aware (via the information sheet and consent form) that should they withdraw, the data already collected up to the time of withdrawal may still be used in the final analysis.

5.1. **Inclusion Criteria**

A patient will be eligible for inclusion in this study only if all of the following criteria apply:

<table>
<thead>
<tr>
<th>AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 18 and above years of age inclusive, at the time of signing the informed consent.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TYPE OF PATIENT AND DIAGNOSIS INCLUDING DISEASE SEVERITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Patients with suspected ILD listed for TBCB for clinical reasons following review by the ILD services at UCLH in whom diagnosis has remained unclear following radiological and clinical assessment.</td>
</tr>
</tbody>
</table>

A patient with a clinical abnormality or laboratory parameter(s) which is/are not specifically listed in the inclusion or exclusion criteria, outside the reference range for the population being studied, may be included only if the investigator in consultation with the Medical Monitor [if required] agree and document that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures.
5.2. **Exclusion Criteria**

A patient will not be eligible for inclusion in this study if any of the following criteria apply:

**CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)**

1. Patients who have a known drug allergy or other contra-indication to ipratropium bromide

**CONTRAINDICATIONS**

2. Known hypersensitivity to atropine or ipratropium bromide or any other known drug allergies that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.

**DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA**

3. As a result of the medical history, physical examination or screening investigations, the physician responsible considers the patient unfit for the study.
4. The patient is unable or unwilling to perform study assessments and procedures correctly.
5. Patients with a recognised co-existing respiratory disorder (other than ILD) that in the opinion of the investigator would confound the study outcomes.
6. Forced expiratory volume in 1 second (FEV1) predicted <65%

5.3. **Screening/Baseline Failures**

Screen failures are defined as subjects who consent to participate in the clinical trial but never subsequently donate a sample for research. All screen failures will be entered into the eCRF.

In order to ensure transparent reporting of screen failure subjects, which meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, a minimal set of screen failure information is required including demography, screen
failure details, eligibility criteria, and serious adverse events. This is also required for response to queries from regulatory authorities.

5.4. Withdrawal/Stopping Criteria

A patient may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons. If a patient withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records. The patients will be made aware that this will not affect their future care. Patients will be made aware (via the information sheet and consent form) that should they withdraw, the data collected may still be used in the final analysis.

The following actions must be taken in relation to a patient who fails to attend the clinic for a required study visit:

- The site must attempt to contact the patient and re-schedule the missed visit as soon as possible.
- The site must counsel the patient on the importance of maintaining the assigned visit schedule and ascertain whether or not the patient wishes to and/or should continue in the study.
- In cases where the patient is deemed ‘lost to follow up’, the investigator or designee must make every effort to regain contact with the patient (where possible, 3 telephone calls and if necessary a certified letter to the subject’s last known mailing address or local equivalent methods). These contact attempts should be documented in the subject’s medical record.
- Should the patient continue to be unreachable, only then will he/she be considered to have withdrawn from the study with a primary reason of “lost to follow-up”.

5.5. Patient and Study Completion

A completed patient is one who has given consent, and successfully completed all study activities stated in this protocol.

The end of the study is defined as the last subjects last visit.
6. STUDY TREATMENT

Study treatment dosage and administration details are listed in Section 6.1

6.1. Other Study Treatment

The term ‘study treatment’ is used throughout the protocol to describe any combination of marketed products received by the patient as per the protocol design.

<table>
<thead>
<tr>
<th>Study Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product name:</strong></td>
</tr>
<tr>
<td>ipratropium bromide</td>
</tr>
<tr>
<td><strong>Formulation description:</strong></td>
</tr>
<tr>
<td>0.025% w/v ipratropium bromide</td>
</tr>
<tr>
<td><strong>Dosage form:</strong></td>
</tr>
<tr>
<td>Colourless nebuliser solution</td>
</tr>
<tr>
<td><strong>Unit dose strength(s)/Dosage level(s):</strong></td>
</tr>
<tr>
<td>500 mcg or 250 mcg</td>
</tr>
<tr>
<td><strong>Route of Administration:</strong></td>
</tr>
<tr>
<td>Inhaled/nebulised</td>
</tr>
<tr>
<td><strong>Dosing instructions:</strong></td>
</tr>
<tr>
<td>500 mcg nebulised according to standard clinical procedures at UCLH prior to the cryobiopsy procedure for 10 minutes.</td>
</tr>
<tr>
<td><strong>Physical description:</strong></td>
</tr>
<tr>
<td>Clear colourless solution in white plastic ampoules.</td>
</tr>
<tr>
<td><strong>Device:</strong></td>
</tr>
<tr>
<td>Commercially available nebuliser</td>
</tr>
<tr>
<td>Or pressurised nebulised air at 10 l/minute</td>
</tr>
<tr>
<td><strong>Method for individualizing dosage:</strong></td>
</tr>
<tr>
<td>A single dose of 500 mcg will be administered to all participating subjects from hospital stock at UCLH.</td>
</tr>
</tbody>
</table>

Sedatives and other anaesthetic agents will be used as per UCLH and British Thoracic Society (BTS) guidelines for diagnostic flexible bronchoscopy for adults.
6.2. **Medical Devices**

Medical devices (not manufactured by or for GSK) for use in this study are:

- Nebuliser.
- ERBE bronchoscopy probe
- Flexible bronchoscope

Medical device use will be in accordance with UCLH standard operating procedures for flexible bronchoscopy.

Instructions for medical device use are as provided by the device manufacturer.

6.3. **Treatment Assignment**

No treatment assignment is required. All subjects consenting to the study will receive nebulised ipratropium bromide prior to the cryobiopsy procedure. Ipratropium bromide will be administered in accordance with the European Summary of Product Characteristics (SPC).

6.4. **Blinding**

There will be no blinding as part of this study. All subjects will be given nebulised ipratropium bromide and will undergo the cryobiopsy procedure as part of their clinical diagnostic testing.

6.5. **Packaging and Labeling**

The contents of the label will be in accordance with all applicable regulatory requirements. Ipratropium bromide nebuliser solution will be supplied in sterile unit dose ampoules in the packaging supplied by the manufacturer.

6.6. **Preparation/Handling/Storage/Accountability**

No special preparation of the study treatment is required; ipratropium bromide will be dosed according to the European SPC and UCLH requirements.

Only authorized site staff will supply or administer study treatment. All study treatments must be stored in a secure environmentally controlled and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorized site staff.

Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff. Site staff should take adequate precautions to avoid direct eye or skin contact and the generation of aerosols or mists. In the case of unintentional occupational exposure, site staff should notify the Medical Monitor and/or GSK study contact.

Handling of study treatment will be done according to UCLH recommended instructions/precautions, which is in line with manufacturer’s instructions.
6.7. Compliance with Study Treatment Administration

Ipratropium bromide is a well established commercially available bronchodilator that is routinely used in respiratory clinical settings.

In this study subjects will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study treatment and study patient identification will be confirmed at the time of dosing by a member of the study site staff. The subjects will then undergo a bronchoscopy and TBCB.

6.8. Treatment of Study Treatment Overdose

The only medication to be administered as part of the study is ipratropium bromide; administration will be done by suitably qualified staff according to local hospital procedures. Only one dose of study treatment will be administered per patient during the study therefore potential to overdose is considered to be highly unlikely.

In the unlikely event of an overdose the investigator or treating physician should:

1. Contact the Medical Monitor immediately
2. Closely monitor the patient for adverse events (AEs)/serious adverse events (SAEs) as per local hospital procedures
3. Document the quantity of excess dose as well as the duration of the overdosing in the CRF.

All other treatments provided during the procedure will be handled according to the local UCLH Standard Operating Procedure (SOP).

6.9. Treatment after the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because it is not expected that the study treatment will provide any therapeutic benefit to the subjects. A single dose of ipratropium bromide will be used to allow imaging of inhaled drug distribution in the lung interstitium of ILD patients. However following diagnosis patients will receive routine treatment from UCLH as per local procedures.

The investigator is responsible for ensuring that consideration has been given to the post-study care of the subject’s medical condition, whether or not GSK is providing specific post-study treatment.
6.10. Lifestyle and/or Dietary Restrictions

6.10.1. Meals and Dietary Restrictions

Patients are to have nil by mouth from midnight prior to the cryobiopsy procedure, as per standard practice for patients having bronchoscopy.

6.11. Concomitant Medications and Non-Drug Therapies

Subjects will be allowed to continue taking their medication as per normal practice for patients having TBCB.

A list of all concomitant medications for co-morbid conditions taken up to 48 hours prior to the TBCB procedure will be recorded in the eCRF. The minimum requirement is that drug name and if possible the dates and time of administration are to be recorded.

6.11.1. Permitted Medications and Non-Drug Therapies

Patients will be permitted to continue taking their medication and non drug therapies as per clinical routine practice for patients having a cryobiopsy procedure with the exception of medications listed in Section 6.11.2.

6.11.2. Prohibited Medications and Non-Drug Therapies

Anticoagulants; subjects taking anticoagulants will be required to safely stop the medication as directed by their doctor according to BTS guideline for diagnostic flexible bronchoscopy in adults.

7. STUDY ASSESSMENTS AND PROCEDURES

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential and required for study conduct. This section lists the procedures and parameters of each planned study assessment. Detailed procedures for obtaining each assessment are provided in the SRM. The exact timing of each assessment is listed in the Time and Events Table Section 7.1

- The timing and number of planned study assessments may be altered during the course of the study based on newly available data to ensure appropriate monitoring.
- The exact timing for each assessment will be recorded in the eCRF.
- Any change in timings will be captured as a protocol deviation. Additional assessments will be recorded as unscheduled assessments and entered in the eCRF.
- The Institutional Review board (IRB)/ Independent Ethics Committee (IEC) will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form.
### Time and Events Table

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Screening (up to 6 weeks prior to the day of procedure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Treatment Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed consent</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

#### Notes

- ECG Remedy
- Post Current Medication
- Lung Function (include FVC and FEV1)
- Vital Sign (include FEV1 and FVC)
- Laboratory assessments (include liver function tests and hematology)
- Medical History
- Brief physical exam
- Demographics
- Inclusion and exclusion criteria
- Clinical outcomes

#### Procedure

<table>
<thead>
<tr>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(up to 7 days post-procedure)</td>
<td>(Follow-up)</td>
<td>(Follow-up)</td>
<td>(Follow-up)</td>
<td>(Follow-up)</td>
</tr>
</tbody>
</table>

#### 7.1.

**CONFIDENTIAL**

2015N221103-00
<table>
<thead>
<tr>
<th>Procedure</th>
<th>Screening (up to 6 weeks prior to the day of procedure)</th>
<th>Treatment Period [hours]</th>
<th>Follow-up (7-14 days post-procedure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notes</td>
<td>Patient will recover in the designated recovery area as per local SOP</td>
<td>0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

**Notes:**
- Procedure
- Chest X-Ray
- AEaSAE review
- Procedure review

**AEaSAE review**
- Procedure
- Patient will recover in the designated recovery area as per local SOP

**Ches:**
- X-Ray
7.2. **Demography and Medical History Assessments**

Medical, medication and family history will be assessed as per routine procedures at UCLH.

The following demographic parameters will be captured: year of birth, sex, and ethnicity.

Lung function; the most recent before the TBCB procedure; repeat measures to be done if available lung function has not been done in the last 3 months from day of procedure.

Concomitant medication and diagnosis confirmation will be collected. Smoking history will also be taken.

Procedures conducted as part of the subject’s routine clinical management: High Resolution Computed Tomography (HRCT) and lung function obtained prior to signing of informed consent may be utilised for screening or baseline purposes provided the procedure meets the protocol-defined criteria and has been performed in the timeframe of the study.

7.3. **Safety**

All bronchoscopies will be carried out under deep sedation using a propofol infusion administered by a qualified anaesthetist at UCLH. Oxygen will be insufflated continuously through nasal cannula; spontaneous respiration will be maintained. Oxygen saturation, blood pressure, ECG and expired carbon dioxide will be monitored continuously. Planned time points for all safety assessments are listed in the Time and Events Table (Section 7.1). Additional time points for safety tests such as vital signs, physical exams and laboratory safety tests may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

The study team including the investigator and GSK medical monitor will have regular meetings to review study progress and safety of study patients.

7.3.1. **Adverse Events (AE) and Serious Adverse Events (SAEs)**

The definitions of an AE or SAE can be found in Appendix 1

The occurrence of adverse events as a result of this study will be recorded in the eCRF by the study investigator or appropriately qualified designee and will be reported to the study sponsor.

The investigator and their designees are responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

7.3.1.1. **Time period and Frequency for collecting AE and SAE information**

Any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) will be recorded from the time a patient consents to participate in the study up to and including any follow-up contact.
AEs will be collected from the start of study treatment until the follow-up contact at the time points specified in the Time and Events Table (Section 7.1).

Medical occurrences that begin prior to the start of study treatment but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the eCRF.

All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Appendix 1.

Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a patient has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

NOTE: The method of recording, evaluating and assessing causality of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Section 7.3.1.2 (Method of Detecting AEs and SAEs).

7.3.1.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the patient is the preferred method to inquire about AE occurrence. Appropriate questions include:

- “How are you feeling?”
- “Have you had any (other) medical problems since your procedure

After the initial AE/SAE report, the investigator is required to proactively follow each patient at subsequent visits/contacts. All SAEs, and non-serious AEs of special interest (Appendix 1) will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the patient is lost to follow-up. Further information on follow-up procedures is given in Appendix 1.

7.3.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each patient at subsequent visits/contacts. All SAEs, and non-serious AEs of special interest (as defined in Appendix 1) will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the patient is lost to follow-up (as defined in Section 5.4). Further information on follow-up procedures is given in Appendix 1.

7.3.1.4. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as SAEs

The following disease related events (DREs) are common in subjects with ILD and can be serious/life threatening:

- Event A - Acute Exacerbation
Because these events are typically associated with the disease under study, they will not be reported according to the standard process for expedited reporting of SAEs to GSK (even though the event may meet the definition of a SAE). These events will be recorded on the DRE page in the subject’s eCRF. These DREs will be monitored by the study team including the Investigator and GSK Medical Monitor on a routine basis.

**NOTE:** However, if the following condition applies, then the event must be recorded and reported as an SAE (instead of a DRE):

*The event is, in the investigator’s opinion, of greater intensity, frequency, or duration than expected for the individual subject*

### 7.3.1.5. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to GSK of SAEs related to study treatment (even for non-interventional post-marketing studies) is essential to fulfill legal obligations and ethical responsibilities towards the safety of subjects. GSK has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. GSK will comply with country specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Board (IRB)/Independent Ethics Committee (IEC) and investigators.

Investigator safety reports are prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and GSK policy and are forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from GSK will file it with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

### 7.3.2. Physical Exams

A brief physical examination will include, at a minimum, assessments of the lungs and cardiovascular system. Height and weight will also be recorded at screening.

Investigators should pay special attention to clinical signs related to previous serious illnesses.

### 7.3.3. Vital Signs

Vital signs will include oxygen saturations, systolic and diastolic blood pressure, pulse rate and respiratory rate.

### 7.3.4. Electrocardiogram

Continuous cardiac telemetry will be performed during the biopsy procedure.
7.3.5. **Clinical Safety Laboratory Assessments**

Laboratory assessments will be conducted as per standard UCLH requirements prior to the cryobiopsy procedure and are defined in Table 1. All samples will be processed by UCLH local laboratory and the results entered in the eCRF.

Haematology, clinical chemistry and additional parameters to be tested are listed in the table below.

### Table 1  Protocol Required Safety Laboratory Assessments

<table>
<thead>
<tr>
<th>Laboratory Assessments</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematology</td>
<td>Platelet Count</td>
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<tr>
<td></td>
<td>RBC Count</td>
</tr>
<tr>
<td></td>
<td>MCV</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
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<td></td>
<td>Haemoglobin</td>
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<td></td>
<td>MCH</td>
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<td></td>
<td>Lymphocytes</td>
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<td></td>
<td>Haematocrit</td>
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<td>Eosinophils</td>
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<td></td>
<td>Monocytes</td>
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<td></td>
<td>Basophils</td>
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<td></td>
<td>Clotting profile tests</td>
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<tr>
<td>Clinical Chemistry</td>
<td>Urea</td>
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<td></td>
<td>Potassium</td>
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<td></td>
<td>Unconjugated bilirubin</td>
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<td></td>
<td>Conjugated bilirubin</td>
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<td>Creatinine</td>
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<td></td>
<td>Sodium</td>
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<tr>
<td></td>
<td>ALP (SGPT)</td>
</tr>
<tr>
<td></td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
</tr>
</tbody>
</table>

7.4. **Trans Bronchial CryoBiopsy Procedure**

Patients who require a TBCB as part of their diagnostic work up will have 500 mcg of ipratropium bromide administered via nebulisation immediately before undergoing bronchoscopy. All bronchoscopies and biopsies will be carried out according to UCLH SOP and a copy has been included in the SRM.

After all clinical samples have been taken, 1 to 2 additional TBCB samples will be taken for research purposes. Up to 5 patients will have in addition to TBCB, endobronchial forceps biopsies taken at the level of the secondary carina to act as a control for drug deposition in the central airways. The exact location of the biopsy will be recorded in the eCRF. Samples taken for research will be processed according to instructions in the SRM.

At 2 hours after the procedure, a chest X-ray will be performed to exclude pneumothorax. Patients will be followed up as per routine care arrangements.

7.4.1. **Sample preparation and analysis procedures**

Cryobiopsy samples taken for research will be embedded in a suitable polymer and processed according to instructions detailed in the SRM. Embedded samples will be transferred to GSK for analysis initially by mass spectrometry techniques, including but
not limited to MALDI-MS, and histology. Co-registration of histology and MSI will be utilised to investigate the distribution of drug in lung samples.

Further exploratory mass spectrometric analyses may be performed, to attempt:

- To identify sites of collagen deposition
- To identify the distribution of collagen subtypes in enzymatically digested tissue sections
- To determine semi-quantitative biopsy drug concentrations
- Metabolomics analysis.

7.5. **Lung Function Measurements/Spirometry**

The most recent lung function performed prior to the trans bronchial cryobiopsy procedure will be recorded in the eCRF as part of this study. A Screening measurement may not be needed if lung function was assessed within 3 months prior to the cryobiopsy. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) will be measured at time points outlined in the Time and Events Table (Section 7.1).

8. **DATA MANAGEMENT**

For this study patient data will be collected from patients in accordance with the protocol, patient consent form and patient information sheet using GSK defined case report forms and combined with data provided from other sources in a validated data system.

Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure integrity of the data, e.g., removing errors and inconsistencies in the data.

Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.

CRFs (including queries and audit trails) will be retained by GSK, and copies will be sent to the investigator to maintain as the investigator copy. Patient initials will not be collected or transmitted to GSK according to GSK policy.

The data will be appropriately sent to the Chief Investigator for statistical analysis, and UCLH will act as the data controller of such data for the study.

The Chief Investigator and Co-Investigators will process, store and dispose of data in accordance with all applicable legal and regulatory requirements, including the Data Protection Act 1998 and any amendments thereto.

The above anonymised data will be entered into a specifically designed database. The database will be password protected and kept on a secure computer at the host institution.

Patient name and address details will be included in the information obtained, but will be kept separate from the medical details. A unique identification number will link the name to the medical details.
The trial personnel, UCLH and any regulatory bodies will keep data confidential. Patient names will not be used in any reports about the study and all data is stored in accordance with the Data Protection Act 1998.

9. **STATISTICAL CONSIDERATIONS AND DATA ANALYSES**

This clinical feasibility study will evaluate the ability of inhaled drugs to reach the lung interstitium by imaging an inhaled drug in trans bronchial cryobiopsies taken from fibrotic regions of the lung of patients undergoing TBCB as part of their diagnostic work up.

Drug distribution in lung interstitial cryobiopsy samples and endobronchial forcep biopsy samples will be determined using our standard operating procedure for MALDI MS Imaging (see SRM). Comparisons will be made of the images both visually and by comparing drug signal intensity. Cryobiopsies from patients not administered ipratropium and supplied for pre-work will serve as control tissue as no compound signal should be detected.

Drug distribution in the biopsy tissue may be correlated with collagen deposition in lung fibrosis. No formal statistical analysis will be taken due to nature of the data expected from this study; mass spectrometry imaging data is semi quantitative.

9.1. **Hypotheses**

No formal hypothesis will be tested; mass spectrometry imaging data can be utilised to image and semi quantitatively measure inhaled interstitial drug deposition in patients with pulmonary fibrosis.

9.2. **Sample Size Considerations**

The sample size is based on feasibility of recruiting subjects in the duration of the study and is considered adequate to achieve the objectives of the study.
10. STUDY GOVERNANCE CONSIDERATIONS

10.1. Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

10.2. Regulatory and Ethical Considerations, Including the Informed Consent Process

The study will be conducted in accordance with all applicable regulatory requirements, and with GSK policy.

The study will also be conducted in accordance with International Conference on Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP), all applicable patient privacy requirements, and the guiding principles of the current version of the Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favorable opinion/approval of the study protocol and amendments as applicable
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)
- GSK will provide full details of the above procedures, either verbally, in writing, or both.
- The IEC/IRB, and where applicable the regulatory authority, approve the clinical protocol and all optional assessments, including genetic research.
- Signed informed consent must be obtained for each patient prior to participation in the study

10.2.1. Urgent Safety Measures

If an event occurs that is related to the conduct of the study and this new event is likely to affect the safety of the patients, the sponsor and the investigator will take appropriate urgent safety measures to protect the patients against any immediate hazard.

The sponsor will work with the investigator to ensure the IEC/IRB is notified.

10.3. Quality Control (Study Monitoring)

In accordance with applicable regulations including GCP, and GSK procedures, GSK monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements.
When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the CRF will serve as the source document.

GSK will monitor the study and site activity to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.
- The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents

10.4. Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance assessment and/or audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study.

In the event of an assessment, audit or inspection, the investigator (and institution) must agree to grant the advisor(s), auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss the conduct of the study, any findings/relevant issues and to implement any corrective and/or preventative actions to address any findings/issues identified.

10.5. Study and Site Closure

Upon completion or premature discontinuation of the study, GSK will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK Standard Operating Procedures.

GSK reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance.

If GSK determines such action is needed, GSK will discuss the reasons for taking such action with the investigator or the head of the medical institution (where applicable). When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action.

If the study is suspended or prematurely discontinued for safety reasons, GSK will promptly inform all investigators, heads of the medical institutions (where applicable) and/or institution(s) conducting the study. GSK will also promptly inform the relevant regulatory authorities of the suspension or premature discontinuation of the study and the reason(s) for the action.

If required by applicable regulations, the investigator or the head of the medical institution must inform the IRB/IEC promptly and provide the reason for the suspension or premature discontinuation.
10.6. Records Retention

Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.

The records must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.

Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.

The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK standards/procedures, and/or institutional requirements otherwise, the retention period will default to 15 years.

The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

10.7. Provision of Study Results to Investigators, Posting of Information on Publically Available Clinical Trials Registers and Publication

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.
11. REFERENCES


12. APPENDICES

12.1. Appendix 1: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.1.1. Definition of Adverse Events

<table>
<thead>
<tr>
<th>Adverse Event Definition:</th>
</tr>
</thead>
<tbody>
<tr>
<td>An AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.</td>
</tr>
<tr>
<td>NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Events meeting AE definition include:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator.</td>
</tr>
<tr>
<td>Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.</td>
</tr>
<tr>
<td>New conditions detected or diagnosed after study treatment administration even though it may have been present prior to the start of the study.</td>
</tr>
<tr>
<td>Signs, symptoms, or the clinical sequelae of a suspected interaction.</td>
</tr>
<tr>
<td>Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae).</td>
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</table>

<table>
<thead>
<tr>
<th>Events NOT meeting definition of an AE include:</th>
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<tbody>
<tr>
<td>Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject’s condition.</td>
</tr>
<tr>
<td>The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject’s condition.</td>
</tr>
<tr>
<td>Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads</td>
</tr>
</tbody>
</table>
to the procedure is an AE.
Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

### 12.1.2. Definition of Serious Adverse Events

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease, etc).

<table>
<thead>
<tr>
<th>Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a. Results in death</strong></td>
</tr>
<tr>
<td><strong>b. Is life-threatening</strong></td>
</tr>
<tr>
<td>NOTE:</td>
</tr>
<tr>
<td>The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.</td>
</tr>
<tr>
<td><strong>c. Requires hospitalization or prolongation of existing hospitalization</strong></td>
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<tr>
<td>NOTE:</td>
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<tr>
<td>In general, hospitalization signifies that the patient has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician’s office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether “hospitalization” occurred or was necessary, the AE should be considered serious.</td>
</tr>
<tr>
<td>Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.</td>
</tr>
<tr>
<td><strong>d. Results in disability/incapacity</strong></td>
</tr>
<tr>
<td>NOTE:</td>
</tr>
<tr>
<td>The term disability means a substantial disruption of a person’s ability to conduct normal life functions.</td>
</tr>
<tr>
<td>This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life</td>
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</tbody>
</table>
functions but do not constitute a substantial disruption.

e. **Is a congenital anomaly/birth defect**

f. **Other situations:**

Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

### 12.1.3. Definition of Cardiovascular Events

**Cardiovascular Events (CV) Definition:**

Investigators will be required to fill out the specific CV event page of the CRF for the following AEs and SAEs:

- Myocardial infarction/unstable angina
- Congestive heart failure
- Arrhythmias
- Valvulopathy
- Pulmonary hypertension
- Cerebrovascular events/stroke and transient ischemic attack
- Peripheral arterial thromboembolism
- Deep venous thrombosis/pulmonary embolism
- Revascularization

### 12.1.4. Recording of AEs and SAEs

**AEs and SAE Recording:**

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in the CRF. It is **not** acceptable for the investigator to send photocopies of the subject’s medical...
records to GSK in lieu of completion of the GSK, AE/SAE CRF page.

There may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all patient identifiers, with the exception of the patient number, will be blinded on the copies of the medical records prior to submission of to GSK.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.

Subject-completed Value Evidence and Outcomes questionnaires and the collection of AE data are independent components of the study.

Responses to each question in the Value Evidence and Outcomes questionnaire will be treated in accordance with standard scoring and statistical procedures detailed by the scale’s developer.

The use of a single question from a multidimensional health survey to designate a cause-effect relationship to an AE is inappropriate.

### 12.1.5. Evaluating AEs and SAEs

#### Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and will assign it to one of the following categories:

- **Mild**: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- **Moderate**: An event that is sufficiently discomforting to interfere with normal everyday activities.
- **Severe**: An event that prevents normal everyday activities. An AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as ‘serious’ when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

#### Assessment of Causality

The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE.

A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

The investigator will use clinical judgment to determine the relationship.

Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study
treatment will be considered and investigated.

The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of his/her assessment.

For each AE/SAE the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, it is very important that the investigator **always make an assessment of causality for every event prior to the initial transmission of the SAE data to GSK**.

The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE data collection tool accordingly.

The causality assessment is one of the criteria used when determining regulatory reporting requirements.

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**Follow-up of AEs and SAEs**

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE.

The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals.

If a patient dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings, including histopathology.

New or updated information will be recorded in the originally completed CRF.

The investigator will submit any updated SAE data to GSK within the designated reporting time frames.

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**12.1.6. Reporting of SAEs to GSK**

**SAE reporting to GSK via electronic data collection tool**

Primary mechanism for reporting SAEs to GSK will be the electronic data collection tool

If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the Medical Monitor.

Site will enter the serious adverse event data into the electronic system as soon as it becomes available.

The investigator will be required to confirm review of the SAE causality by ticking the ‘reviewed’ box at the bottom of the eCRF page within 72 hours of submission of the...
SAE.

After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) will be taken off-line to prevent the entry of new data or changes to existing data.

If a site receives a report of a new SAE from a study patient or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, the site can report this information on a paper SAE form or to the Medical Monitor by telephone.

Contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

### SAE reporting to GSK via paper CRF

Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the Medical Monitor.

In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the SAE data collection tool sent by overnight mail.

Initial notification via the telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.

Contacts for SAE receipt can be found at this beginning of the protocol on the Sponsor/Medical Monitor Contact Information page.
APPENDIX 3- INHALE CONSENT FORM
1. Introduction

You have been provided the information booklet explaining clinical trials. This document is the Informed Consent Form. It contains specific information about this clinical study.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish.

This consent form has been reviewed and approved by an Ethics Committee (EC). This board/committee reviews research studies to protect the rights and well-being of the people taking part. Some of the information in this consent form is required by law.

2. What is “giving your consent”?

Only you can decide if you want to take part in this study. You should only make your decision after reading all the questions and answers in this form.

You may talk to your family, friends and or your family doctor to help make your decision. You can take as much time as you like to decide.

After you have read the entire form, you will be given the chance to ask any questions that you may have. When you have had the chance to ask any questions and they have been answered to your satisfaction, if you decide to take part, sign the consent form. This is called “giving your consent”.
Even after you have signed this study consent form you can change your mind and decide not to participate in the study. You do not have to give a reason.

3. **What is the purpose of the study?**

The purpose of this study is to evaluate the ability of inhaled drugs to reach certain regions of the lung by assessing lung biopsy samples taken by Trans-bronchial Cryo Biopsy (TBCB) procedure after you have inhaled ipratropium bromide. Ipratropium bromide belongs to the group of medicines called bronchodilators which work by expanding the airways of the lung. Ipratropium bromide is widely used in treating lung conditions such as asthma or emphysema which are associated with wheezing and shortness of breath, and has a well-established safety record. In this study, ipratropium bromide will be used as an example of inhaled drug.

Interstitial Lung Diseases (ILDs), such as pulmonary fibrosis are a major cause of illness in the United Kingdom (UK). We are interested in understanding how well patients with ILDs can inhale medicines. This knowledge will help the development of new drugs that are more effective at treating ILDs.

4. **Who is paying for the study and what do they do?**

GlaxoSmithKline (also called “GSK”) is a company that discovers and makes vaccines, medicines and other health products. GSK does not pay the study doctor and UCLH to run this study.

5. **What is involved in the study?**

This study will recruit 10 to 20 patients with suspected fibrotic ILD who are referred for TBCB at University College London Hospital (UCLH) to help make a firm diagnosis.

If you agree to take part in this study, you will have one or two extra TBCB samples taken for research at the same time as your biopsy procedure that is being done as part of your doctor’s clinical management plan. We will also take one to three traditional forceps biopsies from the central main airways of up to 5 patients as part of the study. The samples taken from the central region will be used to compare how well the drug is distributed in a different region of the lung.

No other hospital visits will be required as part of the study.

On the day of the bronchoscopy procedure the following will occur:
You won’t be able to eat anything for six hours before the procedure, but you can take your regular medication with a small amount of water. You will be admitted to the endoscopy or day surgery unit at UCLH before the procedure by nursing staff. They will measure your blood pressure, heart rate and oxygen saturations and weigh you. You will also be seen by the anaesthetist present during the procedure to assess your sedation needs and the respiratory physician performing the procedure will take your consent. You will receive nebulised ipratropium bromide 500 micrograms for 10 minutes before undergoing bronchoscopy.

Once ready, you will be taken to the procedure room.

The anaesthetist will insert a cannula, a small, flexible plastic tube, into one of your veins in your arm in order to be able to administer a sedative during the procedure. The sedative will help you relax and make you go to sleep. It is similar to a general anaesthetic. We will also give you an injection of a medication called tranexamic acid which decreases the bleeding risk from the biopsy. Your oxygen levels and heart rate will be monitored during the procedure.

Your doctor will use local anaesthetic spray to numb your throat. This can taste unpleasant but only lasts for a couple of minutes. Your doctor will pass a flexible telescope (bronchoscope) through your mouth and down into your lungs. We will slide a plastic tube over the scope to keep your airway open and protect your vocal cords during the procedure.

Your doctor will use the bronchoscope to examine your airways (bronchi), and then the cryo-probe tip is passed out of the bronchoscope into smaller bronchi to get biopsies from the outer part of the lung. Your doctor will use the cryo-probe to take samples of lung tissue, using an X-ray machine to determine the location within the lung from which the biopsies are taken.

Your doctor will usually take between three to five biopsies in order to diagnose your condition. The additional one to two TBCB samples needed for this research study will only be taken after all clinical samples have been taken and only if it is safe to do so. The central airway research samples will only be taken after TBCB procedure if safe to do so and are not known to carry any additional risks.

The doctor will provide more information if you are one of the patients selected for the central airway samples.

The biopsy procedure usually takes 45 minutes to an hour. You will have a
Participant Information Sheet Version 03 – 6th Mar 2017

A chest x-ray two hours after the procedure. If the chest x-ray is satisfactory and there have been no complications you can be discharged home after two hours.

Following the procedure, the diagnostic samples will be examined in the UCLH pathology department to determine your diagnosis.

The research samples taken to assess drug distribution in the lung will be sent to GSK for analysis.

Ask the study doctor or nurse if you have any questions about the tests and procedures for the study.

As a routine safety procedure, a member of the research team at UCLH will contact you by telephone up to fourteen days after the procedure to check that you are ok.

6. Do I have to take part?

It is entirely up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the usual care you receive or your access to it.

7. What are the known risks of the study or the side effects of any treatment received?

You will already be having a bronchoscopy and a biopsy for diagnostic purposes.

Bronchoscopy is a frequently performed and generally very low risk procedure. A TBCB has similar risks as a traditional forceps biopsy. The two main risks are:

- **Pneumothorax**, where air escapes into the space around the lung. Often a pneumothorax is small and does not cause any problems. If it is large, your doctor may need to insert a tube (chest drain) in the space around the lung to re-inflate the lung. This happens in less than 1 in 10 procedures.
- **Bleeding** from a biopsy site which is usually minor and stops on its own. It is normal to cough up some streaks of blood for a day or two after the procedure.
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It is also relatively common to have a sore throat after the procedure, which gets better quickly. Developing an infection or worsening of your underlying condition is also a possibility but rare.

Samples for this study will be taken only if, in the professional judgement of the doctor performing the procedure, taking additional samples will not impact your safety. You may have side effects from inhaling ipratropium bromide while in this study. Ask the study doctor if you have any questions about the side effects described here. Please note that most side effects described relate to repeated use of ipratropium bromide and a single administration, as in this study, is likely to have fewer side effects.

Side effects may be mild or severe. The following side effects are common and affect between 1 in 10 to 1 in 100 people:

- Headache, dizziness
- Unexpected tightness of the chest, cough and local irritation
- Dry mouth, diarrhoea, constipation, feeling or being sick

The following side effects are uncommon and affect 1 in 100 to 1 in 1000 people:

- Skin rash, itching of skin, nettle rash (urticaria)
- Blurred vision or difficulty focusing
- Increased pressure in your eyes (glaucoma)

8. What are the possible benefits of taking part?

Taking part in this study may not have direct benefit to you. However, knowledge from this study may help doctors better understand how well people with interstitial lung diseases (ILDs) can inhale drugs and how these drugs are distributed in the fibrotic areas of the lung.

This could help researchers develop drugs that are better at treating patients with ILDs and improve treatment for patients with interstitial lung disease in the future.

9. Are there alternatives to taking part in this study?

You may choose not to take part in this study; this will not affect your usual care you receive or your access to it. If you do decide to take part, you are still free to withdraw anytime without giving a reason.
10. Will I receive payment to be part of this study?

You will not be paid for taking part in this study. When a biopsy is taken as part of the study it is considered a gift for research. None of your donated samples will be sold for commercial benefits.

11. What happens to my lung biopsy samples

If you take part in this study, you will be asked to give lung biopsy samples for future laboratory experiments to investigate the distribution of ipratropium bromide to fibrotic areas of the lung. Similar to information collected in the study, your samples may also be used by GlaxoSmithKline (GSK) or shared by GSK with other companies or universities to better understand interstitial lung disease, other diseases or conditions, or to develop drugs.

Your lung biopsy samples will be given the same, anonymised code as your other study information and kept in locked storage. Anyone who works with your samples will hold the information and results in confidence.

GSK may store your lung biopsy samples taken as part of this study within their research facility for up to 15 years after the end of the study after which time your samples will be destroyed. This will allow scientific research to be conducted in the future as new discoveries are made. You may request destruction of your samples at any time by telling your study doctor.

12. The information held about the research subject

We will collect demographic as well as relevant clinical data including imaging data such as computerized tomography (CT) scans and nuclear medicine scans (Positron emission tomography (PET) scans taken as part of your normal clinical care if applicable.

All information which is collected about you during the course of the study will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address, date of birth and all identifiable information (including patient/hospital/NHS number) removed so that you cannot be identified from it. University College London and University College Hospital will be the organisations involved in collecting the data.

13. What happens to my personal and medical information?

It is very important that your personal and medical information stay
Participant Information Sheet Version 03 – 6th Mar 2017

confidential and secure. GSK will protect your information in accordance with current law.

When you sign this consent form you agree that GSK can use your personal and medical information as described here:

- Your personal and medical information may be checked by GSK and others (like agencies that approve and monitor studies). This is to make sure that the study is being run properly.

- Besides that, only the researchers at this study site can use information that identifies you (such as name and address) and only for the purpose of the study.

- Your study information will be labelled with a code number (for example, 1234782). It will not include your name or address. The study doctor will have the link between your name and the code number.

- The link between your name and the code number will not be shared. Only the code number and coded information will be sent to GSK.

- GSK will use your coded information for research only. This may include research looking at improving the quality and efficiency in conducting clinical research trials in general.

GSK may:

- keep your coded information electronically, and analyse it by computer to find out what the study is telling us. This may be done by GSK or a third party, in which case GSK will ensure that the third party is required to keep your data secure,

- share the information with regulatory agencies that approve new medicines,

- share the information with people who check that the study is done properly (like the ethics committee or review boards),

- combine the information with results from other studies to learn more about other medicines, and this [disease/condition] and other diseases and conditions. This may help us to assess the risks and benefits of GSK (or other) medicines, or to improve disease understanding,

- publish study results for medical journals, meetings and on the internet for other researchers to use; your name will not appear in any publication,
Participant Information Sheet Version 03 – 6th Mar 2017

- share coded information with other companies, organisations or universities to carry out research. This may include research looking at improving the quality and efficiency in conducting clinical research trials in general.

Personal and medical data collected during the study may be moved, stored and used in the country where you live or another country where GSK or those working with GSK work.

Use of this information may take place in countries with lower data protection rules than the country where you live. GSK will make sure that if your data are moved to another country, it will still be treated as stated in this Informed Consent Form.

GSK will be the owner of the study results. GSK plans to use the results, and may get patents or make profits other ways. You will not be paid any part of this.

If you withdraw your consent for use of your personal information, you will no longer be able to continue in the study. However all the information collected before you left the study, or at any follow up visit, will still be used as set out in this consent form. At any time, you may ask the study doctor to see your personal information and correct it, if necessary. In some circumstances, you may not be able to access your study information while the study is ongoing. However, the study doctor will share any important medical information if it is relevant to your health during the course of the study.

14. Do I have to stay in the study?

No. Your participation in the study is voluntary. You may choose to stop taking part in the study at any time, without giving a reason. Inform the study staff if you want to stop being in the study. Your decision will not affect your medical care now or in the future.

15. What happens if I decide to leave the study?

If you decide to leave the study and withdraw your consent, it means you decide that no more information about your health can be collected. You and the study doctor will discuss the best way to do this.
16. What will happen if the findings affect the subject personally?

The diagnostic findings from the bronchoscopic and/or surgical biopsies will form part of your routine clinical care.

It is very unlikely that we will discover something that directly affects you or is relevant to your disease from using the biopsies for laboratory based research.

17. What happens if I get hurt while taking part in this study?

Every care will be taken in the course of this study; the hospital has a duty of care to the participants in the clinical study. However, in the unlikely event that you become ill or are hurt while you are in the study you will get the medical care that you need right away.

GlaxoSmithKline (GSK) may pay you compensation if you are hurt by a medicine or a procedure you needed only as part of this study; GSK holds insurance against claims from participants for injury caused by their participation in this clinical study.

Your study doctor can give you a copy of the guidelines for this kind of injury. After discussing with your research doctor, please make the claim in writing to Dr Porter who is the Chief Investigator for the research and is based at UCLH. Dr Porter will then pass the claim to the Sponsor’s Insurers, via the Sponsor’s office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff or about any side effects (adverse events) you may have experienced due to your participation in the research, the normal National Health Service complaints mechanisms are available to you. Please ask Dr Porter if you would like more information on this. Details can also be obtained from the Department of Health website: http://www.dh.gov.uk

You can also contact our patient advice and liaison service (PALS) at any time at UCLH in the following ways:
1. Post: Ground Floor Atrium, University College Hospital, 235 Euston Road, London NW1 2BU
2. Email to PALS@uclh.nhs.uk
3. Ask a staff member to contact PALS on your behalf and they can visit you in a clinic or in a department.
4. Telephone: 020 3447 3042

You can make a formal complaint to our complaints manager at: Governance Department, UCLH

Signing the consent form does not change any legal rights you may have.

18. **Whom should I call if I have questions?**

You can talk with the study doctor, Dr Joanna Porter about any questions or concerns you have about this study. Call her at [joanna.porter@ucl.ac.uk](mailto:joanna.porter@ucl.ac.uk) or

**Dr Joanna Porter**
Senior Lecturer and Honorary Consultant, University College London Hospitals NHS Trust
Department of Thoracic Medicine
4th Floor East, 250 Euston Road
London NW1 2PG
joanna.porter@ucl.ac.uk
Fax 020 73447 9476
Dear Dr Porter

Study title: Study to assess inhaled drug distribution in the distal lung and interstitium using cryobiopsy samples from subjects with suspected Interstitial Lung Disease undergoing cryobiopsy for clinical reasons

IRAS project ID: 211810
Protocol number: 205053
REC reference: 16/LO/2009
Sponsor: GSK

I am pleased to confirm that HRA Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England
The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. Please read Appendix B carefully, in particular the following sections:

- Participating NHS organisations in England – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- Confirmation of capacity and capability - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria) - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.
Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices
The HRA Approval letter contains the following appendices:
- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval
The document “After Ethical Review – guidance for sponsors and investigators”, issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:
- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:
- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the After Ethical Review document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope
HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.
User Feedback
The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please email the HRA at hra.approval@nhs.net. Additionally, one of our staff would be happy to call and discuss your experience of HRA Approval.

HRA Training
We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is 211810. Please quote this on all correspondence.

Yours sincerely

Rekha Keshvara
Assessor

Email: hra.approval@nhs.net

Copy to: Dr Faron Jordan
Filippo Romanello
Appendix A - List of Documents

The final document set assessed and approved by HRA Approval is listed below.

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contract/Study Agreement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Evidence of Insurance]</td>
<td></td>
<td>17 November 2016</td>
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<tr>
<td>IRAS Application Form [IRAS_Form_25102016]</td>
<td></td>
<td>25 October 2016</td>
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<tr>
<td>IRAS Checklist XML [Checklist_06022017]</td>
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<td>06 February 2017</td>
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<tr>
<td>Notice of Non Substantial Amendment</td>
<td>01</td>
<td>27 June 2017</td>
</tr>
<tr>
<td>Other [Letter of Response to REC]</td>
<td></td>
<td>23 January 2017</td>
</tr>
<tr>
<td>Other [PIS 205053 v2 tracked changes ]</td>
<td></td>
<td>06 January 2017</td>
</tr>
<tr>
<td>Other [ICF v2 tracked changes]</td>
<td></td>
<td>06 January 2017</td>
</tr>
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<td>Participant consent form [Study 205053 Participant Consent form version 03]</td>
<td>03</td>
<td>06 March 2017</td>
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<tr>
<td>Participant information sheet (PIS) [Study 205053 Participant Information sheet version 03]</td>
<td>03</td>
<td>06 March 2017</td>
</tr>
<tr>
<td>Research protocol or project proposal tracked 01</td>
<td></td>
<td>09 June 2017</td>
</tr>
<tr>
<td>Research protocol or project proposal</td>
<td>01</td>
<td>09 June 2017</td>
</tr>
<tr>
<td>Summary CV for Chief Investigator (CI)</td>
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Appendix B - Summary of HRA Assessment

This appendix provides assurance to you, the sponsor and the NHS in England that the study, as reviewed for HRA Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England to assist in assessing and arranging capacity and capability.

For information on how the sponsor should be working with participating NHS organisations in England, please refer to the, participating NHS organisations, capacity and capability and Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria) sections in this appendix.

The following person is the sponsor contact for the purpose of addressing participating organisation questions relating to the study:

Dr Faron Jordan
Email: faron.x.jordan@gsk.com

HRA assessment criteria

<table>
<thead>
<tr>
<th>Section</th>
<th>HRA Assessment Criteria</th>
<th>Compliant with Standards</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>IRAS application completed correctly</td>
<td>Yes</td>
<td>The HRA Approval includes non-substantial amendment 01 dated 27 June 2017.</td>
</tr>
<tr>
<td>2.1</td>
<td>Participant information/consent documents and consent process</td>
<td>Yes</td>
<td>No comments</td>
</tr>
<tr>
<td>3.1</td>
<td>Protocol assessment</td>
<td>Yes</td>
<td>No comments</td>
</tr>
</tbody>
</table>
| 4.1     | Allocation of responsibilities and rights are agreed and documented | Yes | A modified mCTA will act as agreement of an NHS organisation to participate. The following are the main changes made to the original template;  
1. Since GSK was facilitating REC reviews section 4 has been modified to reflect the same |
<table>
<thead>
<tr>
<th>Section</th>
<th>HRA Assessment Criteria</th>
<th>Compliant with Standards</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>The following has been added to 4.2 regarding the disclosure of study summary protocol in a publicly accessible registry. This is a standard GSK requirement. “Trust agrees that such listing shall include a summary of the Protocol, the name of the Investigator at the Trial Site and the details of the institutions conducting the Clinical Trial. Prior to commencement of the Clinical Trial, the Trust shall procure the written consent of the Investigator in respect of disclosure of his or her name in the publicly accessible registry on a worldwide basis”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Reference to regulatory authority has been removed throughout the document as it is not applicable or this study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Reference to investigational medicinal product has been removed since the study drug is not under investigation but a “tool” to assess inhaled drug distribution in the distal lung</td>
<td></td>
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</tr>
<tr>
<td>5.</td>
<td>4.16 added additional standard wording regarding record keeping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>4.17 added information regarding the handling of human biological samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>In appendix 3: a clause 5.5 has been added “If a legal remedy is pursued and the case is the subject of adjudication or settlement, the patient may not bring a further claim, based on the same facts, under these Guidelines”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Appendix 7: GSK anti-bribery and corruption guidelines added</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Section</td>
<td>HRA Assessment Criteria</td>
<td>Compliant with Standards</td>
<td>Comments</td>
</tr>
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</tr>
<tr>
<td>4.2</td>
<td>Insurance/indemnity arrangements assessed</td>
<td>Yes</td>
<td>Where applicable, independent contractors (e.g. General Practitioners) should ensure that the professional indemnity provided by their medical defence organisation covers the activities expected of them for this research study</td>
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<tr>
<td>4.3</td>
<td>Financial arrangements assessed</td>
<td>Yes</td>
<td>The sponsor has confirmed there will be no funding provided to the site. A contract between the sponsor and the site will be used to document site and sponsor responsibilities.</td>
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<td>5.1</td>
<td>Compliance with the Data Protection Act and data security issues assessed</td>
<td>Yes</td>
<td>No comments</td>
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<tr>
<td>5.2</td>
<td>CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed</td>
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<td>No comments</td>
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<td>5.3</td>
<td>Compliance with any applicable laws or regulations</td>
<td>Yes</td>
<td>Human Tissue Act is applicable</td>
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<tr>
<td>6.1</td>
<td>NHS Research Ethics Committee favourable opinion received for applicable studies</td>
<td>Yes</td>
<td>No comments</td>
</tr>
<tr>
<td>6.2</td>
<td>CTIMPS – Clinical Trials Authorisation (CTA) letter received</td>
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<td>No comments</td>
</tr>
<tr>
<td>6.3</td>
<td>Devices – MHRA notice of no objection received</td>
<td>Not Applicable</td>
<td>No comments</td>
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<tr>
<td>6.4</td>
<td>Other regulatory approvals and authorisations received</td>
<td>Not Applicable</td>
<td>No comments</td>
</tr>
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</table>
## Participating NHS Organisations in England

*This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.*

There is only one NHS organisation taking part in the study, there is therefore one type of participating organisation undertaking the research activity as detailed in the study protocol.

The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. For NIHR CRN Portfolio studies, the Local LCRN contact should also be copied into this correspondence. For further guidance on working with participating NHS organisations please see the HRA website.

If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England which are not provided in IRAS or on the HRA website, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net. The HRA will work with these organisations to achieve a consistent approach to information provision.

## Confirmation of Capacity and Capability

*This describes whether formal confirmation of capacity and capability is expected from participating NHS organisations in England.*

Participating NHS organisations in England **will be expected to formally confirm their capacity and capability to host this research.**

- Following issue of this letter, participating NHS organisations in England may now confirm to the sponsor their capacity and capability to host this research, when ready to do so. How capacity and capacity will be confirmed is detailed in the *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* section of this appendix.
- The *Assessing, Arranging, and Confirming* document on the HRA website provides further information for the sponsor and NHS organisations on assessing, arranging and confirming capacity and capability.
Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and the minimum expectations for education, training and experience that PIs should meet (where applicable).

A Principal Investigator is expected to be in place at the participating NHS site.

GCP training is not a generic training expectation, in line with the HRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken.

As a contract commercial study undertaken by local staff, it is unlikely that letters of access or honorary research contracts will be applicable, except where local network staff employed by another Trust (or University) are involved (and then it is likely that arrangements are already in place). Where arrangements are not already in place, network staff (or similar) undertaking any of the research activities listed in A18 or A19 of the IRAS form (except for administration of questionnaires and surveys), would be expected to obtain an honorary research contract from one NHS organisation (if university employed), followed by Letters of Access for subsequent organisations. This would be on the basis of a Research Passport (if university employed) or an NHS to NHS confirmation of pre-engagement checks letter (if NHS employed). These should confirm enhanced DBS checks, including appropriate barred list checks, and occupational health clearance. For research team members only administering questionnaires and surveys, a Letter of Access based on standard DBS checks and occupational health clearance would be appropriate.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England to aid study set-up.

- The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.
- The sponsor has confirmed that the CT and X-Ray are part of standard care and not study related.
Surgical Lung Biopsy in Interstitial Lung Disease

A SYSTEMATIC REVIEW OF THE LITERATURE

OUTLINE PROTOCOL

July 2015

Primary Reviewer: Dr Theresia Mikolasch, University College London, London

Secondary Reviewer: Dr Adam Marshall, Royal Infirmary of Edinburgh, Edinburgh

Background
ILDs are a group of diseases that cause variably progressive scarring of the lungs and lead to significant morbidity and mortality. Many different diseases can result in ILD including connective tissue diseases (CTDs) such as rheumatoid arthritis, drug reactions and allergic responses while other subtypes of ILD are not associated with any triggers and are idiopathic in nature. Frequently the identification of the causative disease or agent can be made from the patient’s history, examination, blood results and radiological findings; however in some cases the underlying disease pattern remains obscure. It is very important to establish the underlying cause as this influences prognosis and treatment. For these reasons a proportion of patients are recommended to undergo a lung biopsy to gain additional histopathological information. Traditionally a lung biopsy is obtained surgically.

Surgical lung biopsies are the current gold standard for obtaining histological material in the diagnosis of IIPs. It is usually performed via the less invasive Video-Assisted Thorascopic Surgical (VATS) approach rather than an open thoracotomy biopsy. Following the 2011 ATS/ERS consensus statement about two thirds of cases of IPF can be diagnosed on the basis of typical radiological findings of UIP and clinical picture. Therefore around a third of patients with suspected IPF would require SLB to confirm or refute the diagnosis. We estimate from two surveys that only 7.5%-12% of suspected IPF patients undergo SLB in the UK which might reflect the reluctance of clinicians to refer patients for a procedure associated with significant mortality and morbidity.

The average hospital stay associated with VATS biopsy is 2-4 days. Mortality has been reported as 3-4% and overall complication rate up to 16%. 57% of patients report pain at the incision site 6-12 months after surgery. Other common complications include persistent air-leak, exacerbations of underlying ILD due to mechanical stress of single lung ventilation, bleeding and delayed wound healing. A 2014 paper from Scotland reported a case series of ILD patients undergoing VATS lung biopsy reported a 1.5% 30 day mortality and complications in 28.8% with an average hospital stay of 3.53 days. A definite pathological diagnosis was reached in 74.2% (other studies range from 34%-100%). This can be regarded as an up to date benchmark for VATS biopsies in the UK.
In 2012, 910 SLB were performed in the UK(43) for suspected ILD at a cost of about £2.7 million; Considering an average hospital stay of about 3.5 days this amounts to 8.7 patient years (PY) spent in hospital. If guidelines were to be followed and 1/3 of cases were biopsied costs would increase to £5million/yr and 16 PY/yr would be spent in hospital; with the projected rise in IIP cases the strain on the NHS will further increase.

Objectives
Primary: To assess morbidity and mortality of surgical lung biopsies in ILD.
Secondary: Length of hospital stay; pathological diagnostic yield; contribution to overall diagnosis; and change of treatment following SLB

Methods
Types of studies
Original research studies and case series published in English evaluating the use of SLB in ILD. Minimum of 20 patients enrolled. Studies will be included if they recorded mortality and/or complication rates.

Types of Participants
Adults only with evidence of Interstitial Lung Disease or Diffuse Parenchymal Infiltrates on imaging

Types of interventions
Surgical lung biopsy will be defined as either open lung biopsy (OLB) or “video-assisted thoracoscopic surgery” (VATS). Studies evaluating non-standard surgical techniques will be excluded such as surgery without general anaesthetic or medical thoracoscopy

Types of outcome
Primary outcome
Complication rate- e.g prolonged air leak; wound infection; bleeding; chronic wound pain; pneumothorax; exacerbation of underlying lung disease; death
Secondary outcomes
Length of stay
Pathological diagnostic yield
Overall diagnostic yield (taking into consideration clinical and radiological information)
Treatment changes following SLB

Search methods
The optimal search strategy for identifying trials in Embase and PubMed will be modified to include MeSH and free-text terms for surgical lung biopsy and searched from 2000 to present. The Cochrane Central Register of Controlled Trials (CENTRAL) will also be searched using a mix of MeSH and free text terms for surgical lung biopsy (2000-present).
Searches will be supplemented by examining the reference lists and citations of identified studies as well as relevant guidelines and reviews to identify further trial reports.
Selection of studies
All relevant abstracts will be assessed by two independent reviewers. Full papers will be obtained, where available, for those deemed potentially eligible, and two reviewers will agree the final set of review papers.

Data extraction and management
Data on study characteristics, patient characteristics, interventions and outcomes will be extracted from publications and presentations using EPPI-Reviewer 4 software.

Study characteristics
- Study size
- Retrospective vs prospective
- Location

Patient characteristics
- Age
- Sex
- Lung Function
  - Pre-biopsy diagnosis

Intervention
- Surgical approach used
- Number of biopsies taken
- Biopsy sites

Outcomes
- Complications
- 90 day mortality
- Length of stay
- pathological diagnosis
  - overall diagnosis
  - change in treatment

Analyses
The studies will be classified, in increasing order of rigor, as retrospective case series without controls, prospective case series without controls, case series with literature controls, case series with historical controls, case series with concurrent controls, case series with concurrent controls assessed by multiple regression
analysis to adjust for important prognostic factors, and randomized controlled trials.
The quality and validity of each study will be assessed using the QualSyst tool for quantitative studies. This tool is based on the study design, method of population sampling, strategies of data collection and analysis, and how the conclusions were ascertained.

Conflicts of interest
No conflicts of interests to declare

Acknowledgements
This review forms part of a PhD thesis.

References

Appendix 1

Search Strategies


Appendix 2

QualSyst Criteria
Table 1. Checklist for assessing the quality of quantitative studies

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<th>Criteria</th>
<th>YES (2)</th>
<th>PARTIAL (1)</th>
<th>NO (0)</th>
<th>N/A</th>
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<tr>
<td>1  Question / objective sufficiently described?</td>
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<td></td>
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<tr>
<td>2  Study design evident and appropriate?</td>
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<tr>
<td>3  Method of subject/comparison group selection or source of information/input variables described and appropriate?</td>
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<td></td>
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<tr>
<td>4  Subject (and comparison group, if applicable) characteristics sufficiently described?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5  If interventional and random allocation was possible, was it described?</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6  If interventional and blinding of investigators was possible, was it reported?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7  If interventional and blinding of subjects was possible, was it reported?</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8  Outcome and (if applicable) exposure measure(s) well defined and robust to measurement / misclassification bias? means of assessment reported?</td>
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<tr>
<td>9  Sample size appropriate?</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10 Analytic methods described/justified and appropriate?</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>11 Some estimate of variance is reported for the main results?</td>
<td></td>
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<tr>
<td>12 Controlled for confounding?</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>13 Results reported in sufficient detail?</td>
<td></td>
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<td></td>
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<tr>
<td>14 Conclusions supported by the results?</td>
<td></td>
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</tr>
</tbody>
</table>

How to calculate the summary score

Total sum = (number of “yes” * 2) + (number of “partials” * 1)

Total possible sum = 28 - (number of “N/A” * 2)

Summary score: total sum / total possible sum
APPENDIX 6 – NLR PROTOCOL
The Neutrophil Lymphocyte Ratio as a prognostic factor in Interstitial Lung Diseases
(student study)

NLR in ILD

Chief Investigator:
Joanna Porter PhD FRCP
Reader in Respiratory Medicine, Leukocyte Trafficking Laboratory
University College London | Rayne Building | 5 University Street | London WC1E 6JF

Supported by:
Breathing Matters

Sponsored by:
University College London Hospitals NHS Foundation Trust (UCLH)

Protocol version number and date:
Version 2, 11/09/2019

R&D / Sponsor Reference Number(s): 18/0145

IRAS Registration Number: 244969
PROTOCOL VERSIONS

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<th>Version Date</th>
<th>Protocol updated &amp; finalised by;</th>
<th>Appendix No</th>
<th>detail the reason(s) for the protocol update</th>
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<td>Current</td>
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<td>11/09/2019</td>
<td>Dr Theresia Mikolasch</td>
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<td>Minor amendment</td>
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DECLARATIONS

The undersigned confirm that the following protocol has been agreed and accepted and that the investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the Research Governance Framework 2005 (as amended thereafter), the Trust Data & Information policy, Sponsor and other relevant SOPs and applicable Trust policies and legal frameworks.

I (investigator) agree to ensure that the confidential information contained in this document will not be used for any other purposes other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I (investigator) also confirm that an honest accurate and transparent account of the study will be given; and that any deviations from the study as planned in this protocol will be explained and reported accordingly.

Signature: Dr Joanna C Porter

Date: 11/09/2019

Print Name (in full): Reader in respiratory medicine and consultant at UCLH
STUDY SUMMARY

<table>
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<td>REC Reference No</td>
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<td>Sponsor Reference No</td>
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<table>
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<tr>
<th>Full (Scientific) title</th>
<th>The Neutrophil Lymphocyte Ratio as a prognostic factor in Interstitial Lung Diseases</th>
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</table>

<table>
<thead>
<tr>
<th>Health condition(s) or problem(s) studied</th>
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<tr>
<td>Idiopathic pulmonary fibrosis (IPF)</td>
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<tr>
<td>Interstitial Lung Disease (ILD)</td>
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<table>
<thead>
<tr>
<th>Study Type i.e. Cohort etc</th>
<th>Retrospective cohort analysis</th>
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<table>
<thead>
<tr>
<th>Target sample size</th>
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<tbody>
<tr>
<td>~200 (initial cohort)</td>
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**STUDY TIMELINES**

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<th>Retrospective study</th>
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<tr>
<td>End of Study definition and anticipated date</td>
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**FUNDING & Other**

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<th>Funding</th>
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<td>Breathing Matters</td>
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UCLH Charity (Registered Number 229771)
UCL Respiratory, Rayne Institute UCL,
5 University Street,
London WC1E 6JF

**KEY STUDY CONTACTS**

<table>
<thead>
<tr>
<th>Chief Investigator</th>
<th>Dr Joanna Porter</th>
<th><a href="mailto:Joanna.Porter@ucl.ac.uk">Joanna.Porter@ucl.ac.uk</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub- Investigator</td>
<td>Dr Theresia Mikolasch</td>
<td><a href="mailto:t.mikolasch@ucl.ac.uk">t.mikolasch@ucl.ac.uk</a></td>
</tr>
</tbody>
</table>

**KEY ROLES AND RESPONSIBILITIES**

**SPONSOR:** The sponsor is responsible for ensuring before a study begins that arrangements are in place for the research team to access resources and support to deliver the research as
proposed and allocate responsibilities for the management, monitoring and reporting of the research. The Sponsor also has to be satisfied there is agreement on appropriate arrangements to record, report and review significant developments as the research proceeds, and approve any modifications to the design.

**FUNDER:** The funder is the entity that will provide the funds (financial support) for the conduction of the study. Funders are expected to provide assistance to any enquiry, audit or investigation related to the funded work.

**CHIEF INVESTIGATOR (CI):** The person who takes overall responsibility for the design, conduct and reporting of a study. If the study involves researchers at more than once site, the CI takes on the primary responsibility whether or not he/she is an investigator at any particular site.

The CI role is to complete and to ensure that all relevant regulatory approvals are in place before the study begins. Ensure arrangements are in place for good study conduct, robust monitoring and reporting, including prompt reporting of incidents, this includes putting in place adequate training for study staff to conduct the study as per the protocol and relevant standards.

The Chief Investigator is responsible for submission of annual reports as required. The Chief Investigator will notify the RE of the end of the study, including the reasons for the premature termination. Within one year after the end of study, the Chief Investigator will submit a final report with the results, including any publications/abstracts to the REC.

**PRINCIPLE INVESTIGATOR (PI):** Individually or as leader of the researchers at a site; ensuring that the study is conducted as per the approved study protocol, and report/notify the relevant parties – this includes the CI of any breaches or incidents related to the study.

**KEY WORDS**
Interstitial lung disease, idiopathic pulmonary fibrosis, neutrophil lymphocyte ratio, prognosis

**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
</tr>
<tr>
<td>AR</td>
<td>Adverse Reaction</td>
</tr>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
</tr>
<tr>
<td>BAL</td>
<td>bronchoalveolar lavage</td>
</tr>
<tr>
<td>CI</td>
<td>Chief Investigator</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>CRO</td>
<td>Contract Research Organisation</td>
</tr>
<tr>
<td>DMC</td>
<td>Data Monitoring Committee</td>
</tr>
<tr>
<td>FBC</td>
<td>Full Blood Count</td>
</tr>
<tr>
<td>FEV1</td>
<td>forced expiratory volume over one second</td>
</tr>
<tr>
<td>FVC</td>
<td>forced vital capacity</td>
</tr>
<tr>
<td>GAfREC</td>
<td>Governance Arrangement for NHS Research Ethics</td>
</tr>
<tr>
<td>GAP score</td>
<td>Gender Age Physiology score</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act of 1996</td>
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</table>
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NLR in ILD Protocol, IRAS : 244969, version 1.0 date (20/02/18)
1 INTRODUCTION

Interstitial lung disease (ILD) is an umbrella term for a group of more than 200 different lung diseases with considerable variation in terms of underlying causes, treatment options and prognosis.

Potentially, the most challenging group are the so call Idiopathic Interstitial Pneumonias (IIPs) which have no known cause or association and are often difficult to differentiate and treat.

In addition, there is considerable variation in the prognosis and treatment options of the different IIPs. Idiopathic Pulmonary Fibrosis (IPF) is the most common of these with a median survival of 3 years causing 5000 deaths/yr in the UK. The incidence increases with age and IPF is more common in men. A UK study reported an 11% increase in the annual incidence of IPF between 1991 and 2003\(^1\). The incidence of IIPs and IPF in particular will continue to rise in the future due to the aging population making it highly relevant to the needs of the NHS, present and future. There is a paucity of management options for these diseases and patient selection for available treatment must be carefully considered. The timing of treatment initiation and referral for lung transplantation depends in part on prognosis which is notoriously difficult in these heterogenous disease groups.

This study will evaluate the use of the Neutrophil-Lymphocyte-Ratio (NLR) as a prognostic biomarker of mortality by first determining the ideal NLR cut-off value in a patient cohort which has already been prospectively followed as part of an observational trial. This cut-off will then be validated in our real-life clinical database patient cohort and compared against and combined with existing prognostic models.
Should these results prove promising we will then evaluate whether NLR predicts response to different disease modifying treatments; the anti-fibrotics, pirfenidone and nintedanib, as well as the immunomodulating antibiotic co-trimoxazole and whether it can be used as a longitudinal marker of treatment response. This will involve the analysis of data from 6 large, prospective, RCT trials.

2 BACKGROUND AND RATIONALE

Interstitial lung disease (ILD) has an incidence of approximately 57/100,000 per year and is associated with significant morbidity. The ILDs consist of a heterogeneous group of diseases with varying amounts of interstitial inflammation and fibrosis, however, there is heterogeneity in outcome, with survival in idiopathic pulmonary fibrosis (IPF) particularly poor. Some patients gradually deteriorate, some undergo stepwise progression, whilst others decline rapidly. Moreover, much of the prognostic data heralds from an era when the criteria for diagnosing IPF were less well and differently defined than at present. Prognostic biomarkers in ILD may inform prognosis and guide management decisions such as timing of lung transplant or initiation of treatment. One of the most commonly used prognostic scoring systems is the GAP score (Gender, Age, Physiology), however it is a relatively static model that has been unable to prospectively predict rapidly deteriorating patients, nor is it helpful in assessing treatment response.

NLR has recently been shown to be an independent prognostic factor in several malignancies and has been evaluated in other inflammatory processes. Moreover, NLR has been shown to predict lung involvement in scleroderma and myositis.
3 OBJECTIVES

3.1 Primary Objective

To evaluate the optimal NLR ratio to define high and low risk groups in different ILD subtypes and determine whether NLR can predict time to death/transplant in different ILDs.

3.2 Secondary Objectives

- To determine whether the addition of NLR to existing risk scoring systems improves mortality prediction
- To determine whether NLR can predict treatment response
- To determine whether NLR can be modified by different therapy interventions
- To determine whether other full blood count parameters (e.g. Mean Platelet Value) also independently predict mortality

4 STUDY DESIGN

This is a retrospective study to evaluate NLR as an independent mortality risk predictor in a derivation cohort of ILD patients. The findings will then be validated in a real-life clinical ILD patient cohort. Independence of NLR compared with established risk scores such as GAP will be assessed via cox regression. A NLR/GAP combination risk model will be formulated.

This will further be validated using the data from the placebo arms of several large RCT cohorts and further patient cohorts from other ILD centres. We will assess NLR as a predictive indicator of treatment response in the RCT treatment arms and determine its value longitudinally as a potential early indicator of treatment response.
5 STUDY SCHEDULE

The derivation cohort data journey will be as follows: study data already collected from the clinical trial “Prognostic value of PET/CT/MRI in patients with Fibrotic Disease: Radiological Organ-Specific Function and Quality of Life Correlation” is held on their study computers at the Nuclear Medicine department in UCLH in an excel format. A research radiographer will check the data for completeness and accuracy. Fully anonymised data will be transferred electronically via nhs.net secure email account to the student researcher. The student researcher will data clean and analyse the data on a secure, password protected UCL desktop located in an access restricted office using the statistics programs STATA and R. Anonymised raw data will only be shared within the research team at UCL consisting of statisticians, the student researcher supervisor and sub-investigators named on this protocol. The data will not be shared outside of UCL.

The validation cohort data journey will be as follows: a specialist nurse, who is part of the clinical team but not the research team, will extract patient data -including patient demographics (hospital number, date of birth, age, sex), diagnosis, past medical history, drug history, date of diagnosis, pulmonary function test results, full blood count results, inflammatory markers (CRP,ESR), smoking status, treatments, date of death, date of last contact alive with services – from the existing clinical database and clinical records into an excel spreadsheet held on a password protected NHS computer at the access restricted department of Thoracic Medicine, 4th floor Euston Road. S/he will then fully anonymise the data by removing date of birth and hospital number and assigning a study ID to each record. This fully anonymised spreadsheet will then be transferred via nhs.net secure email account to the student researcher. The research team will not have access to the non-anonymised data. The student researcher will data clean and analyse the data on a secure, password protected UCL desktop located in an access restricted office using
the statistics programs STATA and R. Data will be held both in excel spreadsheets and in STATA data files. Anonymised raw data will only be shared within the research team at UCL consisting of statisticians, the student researcher supervisor and sub-investigators named on this protocol to ensure quality checks on the data analysis. The data will not be shared outside of UCL.

Data from additional NHS study sites will be treated as outlined below and only fully anonymised data will be transferred securely to UCL for data analysis.

Data journey of the RCT listed in section 8: The sponsor holding the original, anonymised data will transfer it securely (exact arrangements depending on sponsors’ individual protocol) to the student researcher. The student researcher will data clean and analyse the data on a secure, password protected UCL desktop located in an access restricted office using the statistics programs STATA and R. Data will be held both in excel spreadsheets and in STATA data files. Anonymised raw data will only be shared within the research team at UCL consisting of statisticians, the student researcher supervisor and sub-investigators named on this protocol to ensure quality checks on the data analysis. The data will not be shared outside of UCL.

6 CONSENT

Not applicable to this retrospective data analysis. Consent either already obtained as part of clinical trial performed

7 ELIGIBILITY CRITERIA

7.1 Inclusion Criteria

- Patient under the care of the clinical ILD team at UCLH with data recorded in the locally held clinical database
- Participants in any relevant clinical trials with ethics approval for further data analysis (see section 8)
7.2 Exclusion Criteria

- Insufficient patient data for analysis available
- Patients with known haematological disorders affecting Full blood count
- Patients on cytotoxic drugs known to affect Full blood count

8 RECRUITMENT

Patient records will be identified from our existing clinical database as well as records of ILD patients enrolled in the study registered as “Prognostic value of PET/CT/MRI in patients with Fibrotic Disease: Radiological Organ-Specific Function and Quality of Life Correlation”. This is estimated to provide an initial cohort of 200-250 patient records.

Following ethics approval we will apply for access to the anonymised patient data using https://www.clinicalstudydatarequest.com as well as seeking direct permission from the sponsors of the following clinical trials. This will provide a cohort of about 2000 patient records:

- Safety and Efficacy of BIBF 1120 at High Dose in Idiopathic Pulmonary Fibrosis Patients (NCT01335464)
- Safety and Efficacy of BIBF 1120 at High Dose in Idiopathic Pulmonary Fibrosis Patients II (NCT01335477)
- A Randomized, Double-Blind, Placebo Controlled, Phase 3 Study of the Efficacy and Safety of Pirfenidone in Patients With Idiopathic Pulmonary Fibrosis (ASCEND Trial) (NCT01366209)
- A Randomized, Double-Blind, Placebo Controlled, Phase 3 Study of the Safety and Efficacy of Pirfenidone in Patients With Idiopathic Pulmonary Fibrosis (NCT00287729)
• A Randomized, Double-Blind, Placebo Controlled, Phase 3, Three-Arm Study of the Safety and Efficacy of Pirfenidone in Patients With Idiopathic Pulmonary Fibrosis (NCT00287716)
• Treating interstitial pneumonia with the addition of co-trimoxazole (ISRCTN22201583)
• Other REC approved trials in ILD

Other UK ILD centres will provide anonymised patient data to externally validate our initial cohort results. Sites will include The Royal Brompton and Harefield NHS, Royal Papworth NHS Trust, North Bristol NHS Trust, University Hospitals of Leicester NHS Trust, Royal Devon and Exeter NHS Foundation Trust, Taunton and Somerset NHS Foundation Trust. In total around 1000 patient records will be used.

9 STATISTICAL METHODS
The analysis population includes patients from separate cohorts providing a feasibility-validation analysis, in other words in the use of NLR in predicting prognosis will be tested in the original cohort and then in an independent (and external) validation cohort to prove that any findings are reproducible and not the result of sampling bias.
Summary statistics will be used to describe patient characteristics. Survival statistical methods, including cox regression and Kaplan-Meier survival curves, will be used to calculate risk of death/prediction of disease free survival. A $p$ value of less than 0.5 considered statistically significant.

10 PATIENT AND PUBLIC INVOLVEMENT (PPI)
There has been no direct PPI in the design of this retrospective, observational study.
11 FUNDING AND SUPPLY OF EQUIPMENT
Clinical fellows involved in collecting the data are sponsored and covered by existing grants/funding. This includes funding from GSK. Any additional costs to be incurred are minimal (e.g. publication fees) since this is a retrospective analysis of existing data and will be covered by a grand by Breathing Matters.
The study funding has been reviewed by the UCL/UCLH Research Office, and deemed sufficient to cover the requirements of the study. NHS costs will be supported via UCLH and/or the Local Clinical Research Network.

12 DATA HANDLING AND MANAGEMENT
The Primary investigator will ensure that all data is stored on password protected UCLH and UCL computers and will be retained for a period of 10 years.

13 MATERIAL/SAMPLE STORAGE
Not applicable to this study as no biological samples or material will be collected.
This is a retrospective data analysis only.

14 PEER AND REGULATORY REVIEW
The study has been peer reviewed in accordance with the requirements outlined by UCL/UCLH.
This study has been peer reviewed within UCLH, by an independent and relevant peer reviewer on 27.3.2018. The Sponsor has accepted these reviews as adequate evidence of peer review.
15 ASSESSMENT AND MANAGEMENT OF RISK
There are no risks associated with this study since it involves the analysis of retrospective data already collected; there is no additional intervention to subjects planned.

16 RECORDING AND REPORTING OF EVENTS AND INCIDENTS

Not applicable to this retrospective data analysis as no direct patient contact or intervention performed as part of this study.

17 MONITORING AND AUDITING
The Chief Investigator will ensure there are adequate quality and number of monitoring activities conducted by the study team. This will include adherence to the protocol and ensure adequate data quality.

The Chief Investigator will inform the sponsor should she have concerns which have arisen from monitoring activities, and/or if there are problems with oversight/monitoring procedures.

18 TRAINING
The Chief Investigator will review and provide assurances of the training and experience of all staff working on this study. Appropriate training records will be maintained in the study files.

19 INTELLECTUAL PROPERTY
All intellectual property rights and know-how in the protocol and in the results arising directly from the study, but excluding all improvements thereto or clinical procedures developed or used by each participating site, shall belong to UCL. Each participating site agrees that by giving approval to conduct the study at its respective
site, it is also agreeing to effectively assign all such intellectual property rights (“IPR”) to UCL and to disclose all such know-how to UCL, with the understanding that they may use know-how gained during the study in clinical services and teaching to the extent that such use does not result in disclosure of UCL confidential information or infringement of UCL IPR.

20 INDEMNITY ARRANGEMENTS

University College London holds insurance against claims from participants for harm caused by their participation in this clinical study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, if this clinical study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical study. University College London does not accept liability for any breach in the hospital’s duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

21 ARCHIVING

UCL and each participating site recognise that there is an obligation to archive study-related documents at the end of the study (as such end is defined within this protocol). The Chief Investigator confirms that she will archive the study master file at University College London Hospitals NHS Foundation Trust for the period stipulated in the protocol and in line with all relevant legal and statutory requirements. The Principal Investigator at each participating site agrees to archive her respective site’s study documents for 5 years and in line with all relevant legal and statutory requirements.

22 PUBLICATION AND DISSEMINATION POLICY

The results of the study will be presented at national and international conferences, and published in international peer reviewed journals.
23 REFERENCES


24 APPENDICES

No appendices
APPENDIX 7- NLR HRA RESEARCH ETHICS COMMITTEE APPROVAL LETTER
Dear Dr Porter

Study title: The Neutrophil Lymphocyte Ratio as a prognostic factor in Interstitial Lung Diseases
IRAS project ID: 244969
Protocol number: 18/0145
REC reference: 18/LO/0937
Sponsor University College London

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales*, as well as any documentation that has been updated as a result of the assessment.

*In flight studies’ which have already started an SSI (Site Specific Information) application for NHS organisations in Wales will continue to use this route. Until 10 June 2018, applications on either documentation will be accepted in Wales, but after this date all local information packs should be shared with NHS organisations in Wales using the Statement of Activities/Schedule of Events for non-commercial studies and template agreement/Industry costing template for commercial studies.

This is a single site study sponsored by the site. The sponsor R&D office will confirm to you when the study can start following issue of HRA and HCRW Approval.

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.
How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?
The document “After Ethical Review – guidance for sponsors and investigators”, issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:
- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?
You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Name: Joanna Porter
Tel: 020 7679 6972
Email: joanna.porter@ucl.ac.uk

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is 244969. Please quote this on all correspondence.
Yours sincerely,

Natalie Wilson  
Assessor

Email: hra.approval@nhs.net

Copy to:  
Dr Theresia Mikolasch, UCL, Student researcher  
Ms Jessica Broni-Tabi, UCL/UCLH JRO, Sponsor contact  
Mr Cameron Berg, University College London, Lead NHS R&D contact
List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

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<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
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<tr>
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<td>24 July 2017</td>
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<td></td>
<td>03 May 2018</td>
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<tr>
<td>Other</td>
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<tr>
<td>Referee's report or other scientific critique report</td>
<td></td>
<td>09 April 2018</td>
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<tr>
<td>Research protocol or project proposal [protocol]</td>
<td>1</td>
<td>20 February 2018</td>
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<td></td>
<td>01 June 2015</td>
</tr>
<tr>
<td>Summary CV for student</td>
<td></td>
<td>04 December 2017</td>
</tr>
<tr>
<td>Summary CV for supervisor (student research)</td>
<td></td>
<td>01 June 2015</td>
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Summary of assessment

The following information provides assurance to you, the sponsor and the NHS in England and Wales that the study, as assessed for HRA and HCRW Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England and Wales to assist in assessing, arranging and confirming capacity and capability.

Assessment criteria

<table>
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<tr>
<th>Section</th>
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<th>Comments</th>
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<td>1.1</td>
<td>IRAS application completed correctly</td>
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<td>No comments</td>
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<td>2.1</td>
<td>Participant information/consent documents and consent process</td>
<td>Yes</td>
<td>No comments</td>
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<tr>
<td>3.1</td>
<td>Protocol assessment</td>
<td>Yes</td>
<td>No comments</td>
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<tr>
<td>4.1</td>
<td>Allocation of responsibilities and rights are agreed and documented</td>
<td>Yes</td>
<td>This is a non-commercial, single site study taking place in the NHS where the single participating NHS organisation is part of a Joint Research Office with sponsor. Therefore, no study agreements are expected.</td>
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<td>4.2</td>
<td>Insurance/indemnity arrangements assessed</td>
<td>Yes</td>
<td>No comments</td>
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<td>Financial arrangements assessed</td>
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<td>Compliance with the Data Protection Act and data security issues assessed</td>
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<td>No comments</td>
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<td>CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed</td>
<td>Not Applicable</td>
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<td>5.3</td>
<td>Compliance with any applicable laws or regulations</td>
<td>Yes</td>
<td>No comments</td>
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<tr>
<td>6.1</td>
<td>NHS Research Ethics</td>
<td>Yes</td>
<td>No comments</td>
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<td>Assessment Criteria</td>
<td>Compliant with Standards</td>
<td>Comments</td>
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<tr>
<td></td>
<td>Committee favourable opinion received for applicable studies</td>
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<td>6.2</td>
<td>CTIMPS – Clinical Trials Authorisation (CTA) letter received</td>
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<td></td>
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<tr>
<td>6.3</td>
<td>Devices – MHRA notice of no objection received</td>
<td>Not Applicable</td>
<td></td>
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<tr>
<td>6.4</td>
<td>Other regulatory approvals and authorisations received</td>
<td>Not Applicable</td>
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</table>

**Participating NHS Organisations in England and Wales**

*This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.*

This is a non-commercial, single site study. There is one site-type involved in the research. Activities and procedures as detailed in the protocol will take place at participating NHS organisations.

If this study is subsequently extended to other NHS organisation(s) in England or Wales, an amendment should be submitted, with a Statement of Activities and Schedule of Events for the newly participating NHS organisation(s) in England or Wales.

The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England and Wales in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. Where applicable, the local LCRN contact should also be copied into this correspondence.

If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England and Wales which are not provided in IRAS, the HRA or HCRW websites, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net or HCRW at Research-permissions@wales.nhs.uk. We will work with these organisations to achieve a consistent approach to information provision.

**Principal Investigator Suitability**

*This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and Wales, and the minimum expectations for education, training and experience that PIs should meet (where applicable).*
A Principal Investigator (PI) is expected at participating NHS organisations. Sponsor will confirm any training with the research team directly.

GCP training is not a generic training expectation, in line with the HRA/HCRW/MHRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken

No Honorary Research Contracts, Letters of Access or pre-engagement checks are expected for local staff employed by the participating NHS organisations. Where arrangements are not already in place, research staff not employed by the NHS host organisation undertaking any of the research activities listed in the research application would be expected to obtain a Letter of Access.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales to aid study set-up.

The applicant has indicated that they do not intend to apply for inclusion on the NIHR CRN Portfolio.
APPENDIX 8 - LUNG COOL Protocol
LUNG COOL TRIAL
CryOextractiOn of Lung tissue for diagnosis of interstitial LUNG disease

Version: 1.0
Date: 13/08/2015
NCT number: TBC
GENERAL INFORMATION

This document describes the LUNG COOL Trial and provides information about procedures for entering patients into it. The protocol should not be used as an aide-memoire or guide for the treatment of other patients. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial.

COMPLIANCE

The trial will be conducted in compliance with the approved protocol, the Declaration of Helsinki (2013, Fortaleza, Brazil), the principles of Good Clinical Practice (GCP), Commission Directive 2005/28/EC with the implementation in national legislation in the UK by Statutory Instrument 2004/1031 and subsequent amendments, the UK Data Protection Act, and the National Health Service (NHS) Research Governance Framework for Health and Social Care (RGF).

SPONSOR

University College London Hospital Trust
Joint Research Office
1st Floor of Maple House
149 Tottenham Court Road
London W1T 7DN
Contact: Tabitha.kavoi@uclh.nhs.uk
Telephone 020 3447 5557
Fax 020 73447 9937

FUNDING

This trial is supported through funding by the charity Breathing Matters as well as GSK funding for a dedicated research fellow. Further funding from Dunhill Medical Trust has been applied for.

TRIAL REGISTRATION

This trial will be registered with the clinicaltrials.gov Clinical Trials Register, following ethics board approval.

SAE REPORTING

Within 24 hours of becoming aware of an SAE, please email a completed SAE form to the Trial Manager on:
Med.lungcool@ucl.ac.uk
TRIAL ADMINISTRATION

Please direct all queries to the Trial Manager at the coordinating site in the first instance; clinical queries will be passed to the Chief Investigator via the Trial Manager.

COORDINATING SITE

UCLH

Switchboard: 020 3456 7890

Email: Med.lungcool@ucl.ac.uk

STAFF

Trial Manager: TBA

Email: Med.lungcool@ucl.ac.uk

Data Manager: TBA

Email: Med.lungcool@ucl.ac.uk

Trial Physician:

Dr Joanna Porter

Tel: 

CHIEF INVESTIGATOR

Dr Joanna Porter MA PhD FRCP
Senior Lecturer and Honorary Consultant, University College London Hospitals NHS Trust
Department of Thoracic Medicine
4th Floor East, 250 Euston Road

CO-INVESTIGATORS

Dr Theresia Mikolasch

UCLH, London, UK

Dr Neal Navani

UCLH, London, UK

Dr Muhunthan Thillai

Papworth Hospital, Cambridge

Eleonore Pablik, Bakk.rex.soc.oec.

Centre for Medical Statistics, Vienna, Austria
## SUMMARY OF TRIAL

<table>
<thead>
<tr>
<th>SUMMARY INFORMATION TYPE</th>
<th>SUMMARY DETAILS</th>
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<tr>
<td>ACRONYM (or Short Title of Trial)</td>
<td>LUNG COOL Trial</td>
</tr>
<tr>
<td>Long Title of Trial</td>
<td>CryOextractOn of Lung tissue for diagnosis of interstitial LUNG disease</td>
</tr>
<tr>
<td>Version</td>
<td>1.0</td>
</tr>
<tr>
<td>Date</td>
<td>13/08/2015</td>
</tr>
<tr>
<td>Study Design</td>
<td>Single arm prospective trial</td>
</tr>
<tr>
<td>Type of Participants to be Studied</td>
<td>Patients with Interstitial Lung Disease (ILD) selected to undergo Video Assisted Thoracic Surgical (VATS) lung biopsy by a specialist ILD Multidisciplinary Team (MDT)</td>
</tr>
<tr>
<td>Setting</td>
<td>Tertiary respiratory and thoracic surgery units - University College London Hospitals</td>
</tr>
<tr>
<td>Interventions to be Compared</td>
<td>This is a single arm trial. However, in order to ascertain that patients included in this study are representative of patients previously referred for VATS lung biopsy, we will collect data on patients who have undergone a VATS lung biopsy for the diagnosis of ILD during the last 2 years at the participating thoracic surgery units.</td>
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<tr>
<td>Study Hypothesis</td>
<td>The use of standard bronchoscopy and transbronchial cryo lung biopsy (TBCLB) will reduce the number of VATS lung biopsies required for the diagnosis of Interstitial Lung Disease and result in healthcare cost savings</td>
</tr>
<tr>
<td>Primary Outcome Measure(s)</td>
<td>Number of VATS lung biopsies avoided and health care cost saved when bronchoscopy and transbronchial cryo lung biopsy are used as first line diagnostic tool for ILD, compared to a pathway using VATS biopsy alone</td>
</tr>
<tr>
<td>Secondary Outcome Measure(s)</td>
<td>1) The sensitivity and specificity of TBCLB for the diagnosis of ILD 2) The diagnostic accuracy of TBCLB for patients with ILD 3) Length of inpatient stay post procedure 4) Complications of TBCLB</td>
</tr>
<tr>
<td>Randomisation</td>
<td>No randomisation required</td>
</tr>
<tr>
<td>Number of Participants to be Studied</td>
<td>66</td>
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<tr>
<td>Duration</td>
<td>24 months</td>
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<tr>
<td>Ancillary Studies/Substudies</td>
<td>Surplus lung biopsy material will be used in laboratory based studies in “An investigation into the mechanisms of lung injury and repair in inflammatory, infective, fibrotic and destructive lung diseases including those associated with autoimmune rheumatic diseases.” REC reference: 13/LO/0900</td>
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<tr>
<td><strong>SUMMARY INFORMATION TYPE</strong></td>
<td><strong>SUMMARY DETAILS</strong></td>
</tr>
<tr>
<td>----------------------------</td>
<td>---------------------</td>
</tr>
</tbody>
</table>
| **Sponsor**                | University College London Hospital Trust  
Joint Research Office  
1st Floor of Maple House  
149 Tottenham Court Road  
London W1T 7DN  
Contact: Tabitha.kavoi@uclh.nhs.uk  
Telephone 020 3447 5557  
Fax 020 73447 9937 |
| **Funders**                | Breathing Matters  
UCLH Charity (Registered Number 229771)  
UCL Respiratory, Rayne Institute UCL,  
5 University Street,  
London WC1E 6JF  
Dunhill Medical Trust Funding application in progress |
| **Trial Manager**          | To be appointed |
| **Chief Investigator**     | Dr Joanna Porter MA PhD FRCP  
Senior Lecturer and Honorary Consultant, University College  
London Hospitals NHS Trust  
Department of Thoracic Medicine  
4th Floor East, 250 Euston Road  
London NW1 2PG  
joanna.porter@ucl.ac.uk  
Work Telephone 020 73447 9004  
Mobile 07753 606633  
Fax 020 73447 9476 |
TRIAL FLOW CHART

COOL LUNG TRIAL

Consecutive ILD patients referred for diagnostic VATS following MDT discussion

Excluded (n=1)
- Not meeting inclusion criteria (n=1)
- Declined to participate (n=1)
- Other reasons (n=1)

TBCLB performed in conjunction with standard bronchoscopy +/- BAL +/- EBUS

Results discussed in ILD MDT – Consensus diagnosis reached?

Yes

Consensus MDT diagnosis

No

VATS lung biopsy

Yes

MDT re-discussion – consensus diagnosis?

No

6 months clinical follow up and re-discussion at MDT
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<th>Expansion</th>
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<td>A&amp;E</td>
<td>Accident and Emergency</td>
</tr>
<tr>
<td>AE</td>
<td>Adverse event</td>
</tr>
<tr>
<td>ANA</td>
<td>Antinuclear antibody</td>
</tr>
<tr>
<td>CF</td>
<td>Consent Form</td>
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<td>CI</td>
<td>Chief Investigator</td>
</tr>
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<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CLRN</td>
<td>Comprehensive Local Research Network</td>
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<td>COP</td>
<td>Cryptogenic Organising Pneumonia</td>
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<tr>
<td>CTD-ILD</td>
<td>Connective Tissue Disease associated Interstitial Lung Disease</td>
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<tr>
<td>CTU</td>
<td>Clinical Trials Unit</td>
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<tr>
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<td>Data Monitoring Committee</td>
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<td>DPA</td>
<td>(UK) Data Protection Act</td>
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<td>Good Clinical Practice</td>
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<td>IDMC</td>
<td>Independent Data Monitoring Committee</td>
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<td>IPF</td>
<td>Idiopathic Pulmonary Fibrosis</td>
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<td>IRAS</td>
<td>Integrated Research Application System</td>
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<td>Institutional Review Board</td>
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<td>MREC</td>
<td>Main Research Ethics Committee</td>
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<td>National Health Service</td>
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<td>Expansion</td>
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<td>NIHR</td>
<td>National Institute for Health Research</td>
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<td>NSIP</td>
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<tr>
<td>NRES</td>
<td>National Research Ethics Service</td>
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<tr>
<td>PALS</td>
<td>Patient Advice and Liaison Services</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>PIS</td>
<td>Patient Information Sheet</td>
</tr>
<tr>
<td>QoL</td>
<td>Quality of life</td>
</tr>
<tr>
<td>QP</td>
<td>Qualified Person</td>
</tr>
<tr>
<td>R&amp;D</td>
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<td>SAR</td>
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<td>Standard deviation</td>
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<td>SLB</td>
<td>Surgical Lung Biopsy</td>
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<td>SOP</td>
<td>Standard operating procedure</td>
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<td>VATS</td>
<td>Video-Assisted Thoracoscopic Surgery</td>
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1 BACKGROUND

1.1 INTRODUCTION

ILDs are a group of diseases that cause variably progressive scarring of the lungs and lead to significant morbidity and mortality. Many different diseases can result in ILD including connective tissue diseases (CTDs) such as rheumatoid arthritis, drug reactions and allergic responses while other subtypes of ILD are not associated with any triggers and are idiopathic in nature. Frequently the identification of the causative disease or agent can be made from the patient's history, examination, blood results and radiological findings; however in some cases the underlying disease pattern remains obscure. It is very important to establish the underlying cause as this influences ability to make an accurate prognosis and treatment decisions. For these reasons a proportion of patients are recommended to undergo a lung biopsy to gain additional histopathological information. Traditionally a lung biopsy is obtained surgically. Advances in bronchoscopy mean that we may be able to get enough information needed by taking lung biopsies in a less invasive way. We plan to investigate whether diagnostic lung biopsies can be taken through a bronchoscope using a cryoprobe. This has been shown to be safe in previous studies and the technique is well established in a number of European centres. UCLH has introduced the technique in 2014.

If we can demonstrate that transbronchial cryoscopic lung biopsy (TBCLB) can establish a pathological diagnosis then fewer patients will require thoracic surgery for diagnosis of ILD sub-type. Surgical lung biopsy is associated with significant mortality and morbidity such as chronic nerve pain as well as significant cost. TBCLB may also increase the use of biopsy in the diagnosis of ILD and make this option available to patients who would not be fit to undergo a surgical procedure. The ultimate aim is that the information gained through histopathological assessment will let us target treatment, predict prognosis, stratify referral for early lung transplant and further biological understanding of the disease.

1.2 INTERSTITIAL LUNG DISEASES

Interstitial lung disease (ILD) is an umbrella term for a group of more than 200 different lung disease and there is considerable variation in underlying causes, treatment options and prognosis. Broadly speaking ILDs can be sub-divided into known causes through exposure such as drug reactions, occupational exposure (e.g. asbestosis), allergen exposure (hypersensitivity pneumonitis) or radiation damage; known causes through association with systemic disease such as ILDs associated with connective tissue diseases (ILD-CTD), sarcoidosis or ILD associated with Inflammatory Bowel disease. Other ILDs are familial or genetic such as familial Idiopathic Pulmonary Fibrosis while others do not fit into clear categories such as lymphangioleiomyomatosis (LAM). Probably the most challenging group are the so call Idiopathic Interstitial Pneumonias (IIPs) which have no known cause or association and are often difficult to differentiate and treat.
There is considerable variation in prognosis and treatment options of different IIPs. Idiopathic Pulmonary Fibrosis (IPF) is the most common with a median survival of 3 years causing 5000 deaths/yr in the UK. The incidence increases with age and IPF is more common in men. A UK study reported an increase of IPF by 11% annually between 1991 and 2003 which was felt not to be attributable to the aging of the population or increased diagnosis. It therefore appears to be a growing problem (2).

The incidence of IIPs and IPF in particular will continue to rise in the future due to the aging population making it highly relevant to the needs of the NHS, present and future. IPF and other ILDs, especially Non-Specific Interstitial Pneumonia (NSIP), are often difficult to distinguish without histological analysis of lung tissue gained through SLB (3-7). There is a paucity of management options for these devastating diseases, but treatments are slowly emerging and accurate diagnosis is increasingly important. Mounting evidence suggests that immunosuppression in NSIP and other fibrotic ILD such as hypersensitivity pneumonitis (HP) with agents such as cyclophosphamide(8) and rituximab(9) is beneficial whereas aggressive immunosuppression in IPF increases mortality(10); the anti-fibrotic agent pirfenidone is the only licensed pharmacological treatment for IPF, but at an annual cost of £26,171 and frequent side effects, accurate patient selection is essential(11).

Since various forms of ILD such as IPF, non-IPF forms of IIP, CTD-ILD, and HP can have similar clinical presentations, patients with suspected ILD must undergo an evaluation that adequately establishes a confident diagnosis of a specific ILD, as treatment and various management decisions are diagnosis-specific and may vary considerably according to the specific form of ILD that is diagnosed.

1.3 DIAGNOSTIC WORK UP IN INTERSTITIAL LUNG DISEASES

Initial diagnostic work up in all newly presenting ILD patients is focused on a thorough clinical history aimed at establishing potential triggers and exposures such as occupational or drug exposure or animal contacts as well as clinical assessment for connective tissue diseases (CTD) and other
associated systemic diseases. This is followed by auto-antibody and serology blood tests to further investigate potential ILD-CTD or exposure to antigens associated with HP such as Aspergillus or Avian antigens.

Full pulmonary lung function testing is also necessary to assess severity of disease and to be able to monitor progress and treatment response.

Echocardiography is also frequently performed to exclude underlying cardiovascular disease which might be contributing to symptoms of dyspnoea and exhaustion. It is also utilised to assess for pulmonary hypertension due to chronic pulmonary disease and to investigate for right-to-left shunts which can exasperate hypoxia.

1.3.1 RADIOLOGICAL WORK UP

Chest Radiographs
Chest radiographs can show a multitude of abnormalities depending on the underlying sub-form of ILD but can on occasion also be normal. Some of the more specific findings identifiable on chest x-ray (CXR) include interstitial infiltrates associated with calcified pleural plaques pointing towards a diagnosis of asbestosis or bilateral hilar lymphadenopathy making sarcoidosis more likely. The distribution pattern of interstitial changes can also be helpful in narrowing down the list of differential diagnosis— for example upper lobe predominance is often associated with chronic HP whereas the lower lobes are mostly affected in IPF or NSIP. Asymmetrical distribution can point towards a degree of gastro-oesophageal reflux and aspiration contributing to interstitial damage. CXRs are rarely sufficient to make a confident diagnosis of a specific ILD but can prompt appropriate further imaging and can be useful in tracking the progression of disease. If previous CXRs are available their review can establish if the interstitial process is chronic or acute.

High Resolution Computed Tomography
High-resolution computed tomography (HRCT) of the thorax is usually a key component of the diagnostic evaluation in suspected ILD. It is nearly universally obtained in patients with suspected ILD and may be diagnostic removing the need for invasive diagnostic approaches such as bronchoscopy or surgical lung biopsy (SLB). The quality of HRCT images and their utility in ILD diagnosis depends on the scanning protocol employed. Traditionally images were obtained at 1 to 2 cm cross-sectional intervals which meant that small focal abnormalities were not visualised and breathing artefacts common. The use of multidetector CT scanners makes it possible to scan the entire thorax in a single breathhold. These images can be reconstructed to contiguous high-resolution images.

The ATS/ERS consensus statement (12) for the diagnosis of IPF set out criteria for the optimal HRCT technique for evaluation of ILD

The scans should be non-contrast and include at a minimum:
- Scans obtained on full inspiration without respiratory motion
- Contiguous or non-contiguous axial scans with thin sections, reconstructed at ≤2 cm intervals
- Reconstructed slice collimation ≤2 mm
- High resolution reconstruction algorithm
- Field of view to include lungs only
- Expiratory scans are helpful to exclude lobular air trapping suggestive of hypersensitivity pneumonitis
- Prone scans if dependent density obscures detail on supine images
- Optional coronal and sagittal reconstructions if volumetric images are obtained
Interpretation of HRCT scans in ILD is based on assessing the extent, specific distribution and severity of the following findings: parenchymal reticulation, centri-lobular nodules, foci of low attenuation, ground-glass attenuation, traction bronchiectasis, air trapping, architectural distortion, and honeycombing. The 2008 Fleischner Society statement (13) has attempted to clarify and standardise the terminology employed when describing HRCT scan findings in ILD but there remains considerable variability and inter-observer variation in diagnosis and confidence of diagnosis. This in part depends on the setting (district hospitals or tertiary referral centres) as well as complexity of the case (14). Sensitivity and specificity of radiological diagnosis also depends on confidence of diagnosis and the nature of the underlying diagnosis. In a retrospective study of NSIP, IPF and chronic HP cases a confident radiological diagnosis was reached in 53% of HRCT readings (n=132) (15).

A definitive Usual Interstitial Pneumonia (UIP) pattern on HRCT has a specificity of approximately 95% and sensitivity of approximately 40% for UIP. In contrast, a predominant feature of ground glass opacities (GGOs) gives a sensitivity of approximately 95% and specificity of approximately 40% for NSIP (16).

Therefore the ATS/ERS consensus statement has set out diagnostic criteria for a definite UIP pattern and recommended that patients with HRCT appearances with possible or inconsistent UIP pattern undergo SLB.

<table>
<thead>
<tr>
<th>UIP Pattern (All Four Features)</th>
<th>Possible UIP Pattern (All Three Features)</th>
<th>Inconsistent with UIP Pattern (Any of the Seven Features)</th>
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<tbody>
<tr>
<td>Subpleural, basal predominance</td>
<td>Subpleural, basal predominance</td>
<td>Upper or mid-lung predominance</td>
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</table>
| Reticular abnormality          | Reticular abnormality                | Lower lobes (blunt, predominantly)
| Honeycombing with or without traction bronchiectasis | Presence of features as inconsistent with UIP pattern (see third column) | Ground-glass abnormality (extent > miliary abnormality)
| Abnormality of features as inconsistent with UIP pattern (see third column) | Diffuse miliary abnormality (upper lobes) | 

Definition of abbreviation: UIP = usual interstitial pneumonia

Table 1: ATS/ERS 2011 Guideline HRCT Criteria for UIP Pattern (12)

Therefore, HRCT appearances consistent with an UIP pattern make the diagnosis of IPF highly likely and additional interventional diagnostics such as bronchoscopy or SLB are unlikely to change the consensus diagnosis. However appearances such as GGOs considered to be more consistent with NSIP have a low specificity and would benefit from SLB.

1.3.2 BRONCHOSCOPY IN THE ASSESSMENT OF ILD

Bronchoalveolar Lavage

Considerable debate and variation in practice surrounds the use of broncho-alveolar lavage (BAL) in the diagnostic work up in ILD. A weak negative recommendation was made against BAL in the majority of patients in the 2011 ATS/ERS IPF diagnostic consensus guidelines but the ensuing controversy led to the ATS publishing guidelines about the role of BAL in ILD diagnosis in 2012. (17) While undoubtedly valuable in excluding infection BAL can only rarely be diagnostic by itself (e.g.
eosinophilic pneumonia); however it can provide valuable additional information when narrowing down the list of possible differential diagnosis. The ATS guidelines concede that BAL cellular analysis may be useful in individuals who lack a confident UIP pattern HRCT. 2013 NICE guidelines for the diagnosis and management of suspected IPF state that BAL might be beneficial in the work up and do not directly refer to HRCT appearances(11). When deciding whether to perform BAL the treating physician has to take into consideration the degree of uncertainty about the type of ILD, the likelihood that the BAL will provide helpful information, and whether the patient would tolerate the procedure as well as patient’s wishes. Recognition of a predominantly inflammatory cellular pattern (increased lymphocytes, eosinophils, or neutrophils) in the BAL differential cell profile frequently helps the clinician narrow the differential diagnosis.

Table 2 Summary of BAL cellular patterns in different ILDs(17)

An interesting study published in 2009 detected a BAL lymphocytosis of >30% in 6 out of 74 patients with definite UIP features on HRCT and in all six cases an alternative diagnosis to IPF was made. Therefore the importance of routine bronchoscopic assessment in suspected IPF remains unclear and recent NICE guidance has included research recommendations into the value of bronchoalveolar lavage (BAL) in the diagnostic pathway(11) BAL is generally well tolerated with an excellent safety profile.

Endobronchial Ultrasound and Transbronchial Needle Aspiration
Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) is a well-established bronchoscopic technique to sample enlarged mediastinal lymph nodes. Lymph node stations accessible by this technique are the paratracheal, subcarinal, hilar and interlobar lymph node stations (2,4,7,10,11). Several fibrosing ILDs can present with a degree of mild mediastinal enlargement which is usually reactive and not actively investigated. The main role of EBUS-TBNA in the ILD diagnostic pathway is to assess larger mediastinal lymphadenopathy in suspected sarcoidosis to exclude alternative pathology such as tuberculosis, lymphoma or other underlying malignancy. It may be performed under conscious sedation and again has an excellent safety profile.

Transbronchial Biopsy with forceps
Transbronchial biopsy (TBBx) utilising standard bronchoscopy forceps is a minimal invasive technique for obtaining lung tissue for histological diagnosis in many pulmonary disorders. However
TBB does not always provide adequate or histologically acceptable lung tissue to set a final diagnosis of heterogeneous lung diseases as the biopsies are small, and subject to crush artefact and may not be representative in spatially heterogenous disease\(^{18, 19}\). It can be helpful in diagnosis of ILDs associated with distinctive histopathological features such as granulomas in sarcoidosis that can be diagnosed on small specimens and do not depend on recognising specific architectural features as in IPF. Berbescu et al showed that the contribution of TBB in diagnosis of IPF is only 32%.\(^{20}\) Worryingly, small sample size and crush artifact in lung tissue acquired during TBB may mislead the pathologist and potentially lead to incorrect diagnoses in the case of IIPs such as IPF and NSIP\(^{21}\). The total number of biopsies taken for optimal diagnostic yield is reported to be 4-10\(^{22}\) The most common complications are pneumothorax (2-6%) and significant bleeding (2-9%) post procedure. The pneumothorax rate may be reduced by using fluoroscopy during the procedure.

**Transbronchial Cryo Lung Biopsy**

Systematic review:
Transbronchial cryo lung biopsy (TBCLB) was first described in 2008\(^{23}\). Since then it has been shown to be a safe, and effective minimal invasive diagnostic tool for the histological diagnosis of ILD, with a diagnostic yield of up to 74–80\%\(^{23-27}\). The advantage of TBCLB over conventional forceps TBB lies in the larger specimen size and lack of crush or bleeding artefacts distorting the tissue architecture.

We performed a literature review as identifying 8 original papers and 4 conference abstracts evaluating TBCLB in patients with ILD. 3 further studies performed CLB in transplant patients\(^{23-37}\). One abstract was excluded from analysis due to insufficient data. These are all case series or feasibility studies, mostly retrospective, with the exception of one prospective randomised trial comparing the safety and efficacy of forceps TBBx to TBCLB. In total 503 cases have been described. The safety profile of TBCLB is comparable to TBBx. Endobronchial bleeding post biopsy was reported in 10% and could be controlled bronchoscopically in all cases. Pneumothorax requiring chest drain insertion occurred in 4%. Exacerbations were described in 3 patients (0.5%) one of whom had a fatal outcome (0.2%). Mean specimen size ranged from 9mm\(^2\) to 64mm\(^2\).

In the prospective randomised trial by Pajares et al, TBCLB mean specimen size was 14.7mm\(^2\) vs 3.3mm\(^2\) for TBBx biopsy (p <0.001). Histological diagnosis was reached in 74.4% of TBCLB group vs. 34.2% in TBBx group respectively (p<0.001)\(^{37}\)

Therefore the large size and preserved architecture of TBCLB make it superior to TBBx. The contribution of CLB obtained histology in the diagnosis of patients with ILD may therefore reduce the number of SLB required to reach a confident diagnosis. To date this has not been evaluated.

**1.3.3 SURGICAL LUNG BIOPSY**

Surgical lung biopsies (SLB) are the current gold standard for obtaining histological material in the diagnosis of IIPs. It is usually performed via the less invasive Video-Assisted Thorascopic Surgical (VATS) approach rather than an open thoracotomy biopsy. Following the 2011 ATS/ERS consensus statement about two thirds of cases of IPF can be diagnosed on the basis of typical radiological findings of UIP and clinical picture. Therefore around a third of patients with suspected IPF would require SLB to confirm or refute the diagnosis. We estimate from two surveys that only 7.5%–12% of suspected IPF patients undergo SLB in the UK\(^ {38, 39}\). This may reflect the reluctance of clinicians to refer patients for a procedure associated with significant mortality and morbidity.
Figure 2: ATS/ERS Diagnostic algorithm for idiopathic pulmonary fibrosis (IPF).
Patients with suspected IPF should be carefully evaluated for identifiable causes of ILD. In the absence of an identifiable cause for ILD, an HRCT demonstrating UIP pattern is diagnostic of IPF. In the absence of UIP pattern on HRCT, IPF can be diagnosed by the combination of specific HRCT and histopathological patterns. The accuracy of the diagnosis of IPF increases with multidisciplinary discussion (MDD) among ILD experts. *Refer to Table 1 for definition. (12)

The average hospital stay associated with VATS biopsy is 2-4 days(40). Mortality has been reported as 3-4% and overall complication rate up to 16%(41). 57% of patients report pain at the incision site 6-12 months after surgery (42). Other common complications include: persistent air-leak; exacerbations of underlying ILD due to mechanical stress of single lung ventilation; bleeding and delayed wound healing.

A 2014 paper from Scotland reported a case series of ILD patients undergoing VATS lung biopsy reported a 1.5% 30 day mortality and complications in 28.8% with an average hospital stay of 3.53 days. A definite pathological diagnosis was reached in 74.2% (other studies range from 34%-100%).(40) This can be regarded as an up to date benchmark for VATS biopsies in the UK.

In 2012, 910 SLB were performed in the UK(43) for suspected ILD at a cost of about £3 million; considering an average hospital stay of about 3.5 days this amounts to 8.7 patient years (PY) spent in hospital. If guidelines were to be followed and 1/3 of cases were biopsied costs would increase to £5million/yr and 16 PY/yr would be spent in hospital; with the projected rise in IIP cases the strain on the NHS will further increase.

1.3.4 MULTIDISCIPLINARY TEAM

No single diagnostic test can be considered to provide a definite, confident diagnosis in almost all ILDs. Therefore a consensus diagnosis reached by a Multidisciplinary Team (MDT) with expertise in ILD is now considered the gold standard. The MDT can integrate all available data at several stages of the diagnostic work up. This does not only improve inter-observer agreement and diagnostic confidence(44) but may also prevent unnecessary surgical biopsies and identify patients in whom a
biopsy may effectively contribute to the diagnosis. Current NICE guidelines recommend that IPF should only be diagnosed by MDT consensus(11). It also recommends a minimum MDT composition of one consultant respiratory physician, one consultant radiologist, an ILD specialist nurse and an MDT co-ordinator all of whom should have expertise in ILD. When invasive diagnostics are considered a consultant histopathologist and, if appropriate, a consultant thoracic surgeon should also form part of the MDT. There is some evidence that involving more than one clinician of the same specialty in a MDT increases inter-observer agreement between specialties(45).
2 TRIAL DESIGN

2.1 HYPOTHESIS

The use of transbronchial cryo lung biopsy in combination with standard bronchoscopy will reduce the number of VATS lung biopsies required for the diagnosis of Interstitial Lung Disease and result in healthcare cost savings.

2.2 PRIMARY OUTCOME MEASURE

Proportion of VATS lung biopsies saved and health care cost savings when using TBCLB as first line diagnostic tool compared to VATS biopsy alone.

2.3 SECONDARY OUTCOME MEASURES

1) The sensitivity and specificity of TBCLB for the diagnosis of ILD
2) The diagnostic accuracy of TBCLB for patients with ILD
3) Length of inpatient stay post procedure
4) Complications of TBCLB

2.4 SUMMARY OF TRIAL DESIGN

This is a single arm trial to evaluate the effectiveness and cost-effectiveness of TBCLB and standard bronchoscopy (including bronchoalveolar lavage (BAL) and EBUS TBNA if appropriate) in reducing the number of VATS SLB, which is currently considered the gold standard investigation for obtaining histopathological samples. Comparison of the outcomes (proportion of patients undergoing VATS SLB and associated cost) will be made with patients who have previously all undergone VATS SLB. Since the proportion of patients in the control arm that undergo VATS SLB is already known (100%), a control arm is not required. Nevertheless all patients fulfilling the inclusion criteria and who underwent VATS SLB in the participating centers between September 2013 to September 2015 will serve as retrospective control group, in order to document the frequency of complications and adverse events for VATS SLB and to estimate the costs and the length of stay for VATS patients.

The trial will involve 66 patients and will take place in University College London Hospital (UCLH).

2.5 METHODOLOGY AND TRIAL INTERVENTION

Participants will be newly referred patients over 18 years old, with evidence of ILD on High Resolution Computed Tomography (HRCT) who are deemed to require a SLB for diagnosis. All participants will undergo clinical assessment by an ILD specialist. They will then be discussed at a specialist ILD MDT (Multidisciplinary team meeting). Patients with known causes for their ILD will be excluded. An experienced respiratory radiologist will review each HRCT. Patients will be offered trial enrolment if following MDT discussion no confident clinical diagnosis can be reached and
histopathology is deemed useful and they have no contra-indications to bronchoscopic TBCLB and VATS SLB. The intervention will involve a bronchoscopy with transbronchial cryoscopic lung biopsy (3-5 samples) of lung parenchyma under fluoroscopy and deep sedation. Standard BAL and EBUS TBNA might also be carried out if clinically indicated. All the trial interventions are currently offered as part of standard care to patients at UCLH.

The patients will receive deep sedation with intravenous propofol and remifentanil by an anaesthetist. The patient will be pre-medicated with 500mg to 1000mg of IV tranexamic acid if no contraindications exist. Oxygen will be insufflated continuously through nasal cannula; spontaneous breathing will be maintained throughout the whole procedure. Oxygen saturation, blood pressure, ECG and transcutaneous carbon dioxide partial pressure will be monitored continuously. The bronchopulmonary segments for biopsy will be determined before the procedure according to the chest CT scan. The patient will be intubated with an uncuffed, re-enforced endotracheal tube (ET)(Bronchoflex, RUSCH). An interventional, flexible bronchoscope will be passed through the ET tube to the pre-selected bronchopulmonary segment. A 5ml aliquot of 1:100000 dilution of adrenaline will be administered to the chosen segment. Following this a flexible cryoprobe (Erbe, CE 0124) measuring 90cm in length and 2.4mm in diameter will be passed through the bronchoscope. Under fluoroscopic guidance, the biopsy instrument will be navigated towards the selected area aiming to keep a distance of 10-15mm from the pleura. The probe will be cooled with nitrous oxide so the temperature in the probe’s tip will decrease to -89°C for 3-5 seconds. Then the cryoprobe and bronchoscope are simultaneously retracted, retrieving the sample frozen to the tip of the probe. The frozen specimens will be thawed in saline and then fixed in formalin. Each patient will have 3-5 biopsies; the exact number will be determined by the operator according to size of the obtained specimens and any complications arising.

At 2 hours after the procedure a chest X-ray will be performed to exclude pneumothorax. The length of the procedure will be measured as the time from starting the propofol infusion to it being switched off. The intensity of bleeding will be assessed as controllable by suction through the flexible bronchoscope or requiring further interventions such as tamponade, instillation of cold saline or adrenaline.

If appropriate a bronchoalveolar lavage (BAL) will be obtained in the standard fashion using a flexible bronchoscope passed to a radiologically predetermined lobe. 60-120ml of sterile 0.9% saline are then injected through the working channel of the bronchoscope and re-aspirated into a sample pot. If appropriate EBUS will be performed as per standard clinical procedure.

Histological specimens will then be processed as per usual process in the local department of pathology according to standard protocols with serial sectioning. Pathologists with experience in examining samples of lung parenchyma of ILD patients will report the pathology with knowledge of the clinical scenario, which closely reflects clinical practice. However, they will be blinded to the fact that patient is in a clinical trial, minimising observer bias. Due to the difference in the samples size produced from TBCLB and VATS SLB it is not possible to blind the pathologists to the procedure employed.

Further size assessment will be carried out using digital imaging software and the area of alveolar tissue of the specimen will be calculated. If a histopathological diagnosis is reached this will be recorded.

All patients will then be re-discussed in the specialist ILD MDT to ascertain firstly a definitive pathological diagnosis and secondly a consensus MDT diagnosis. There will be a proportion of patients in whom a confident pathological and consensus diagnosis cannot be reached. These patients will then be offered VATS SLB. VATS is performed by specialist thoracic surgeons at the
Heart Hospital according to standard clinical practice. This is done under general anaesthesia with single lung ventilation through a double lumen endotracheal tube. This allows deflation of the lung from which the biopsy will be taken. Once the patient has been correctly positioned one or two incisions are made in the intercostal space to allow insertion of the operating instruments. Usually 1 to 3 biopsies are taken from one or two lobes. Following this a large bore chest drain is inserted to allow full re-expansion of the lung. The procedure takes between 60-90 minutes. Conversions to open thoracotomy are very rare. The chest drain is usually removed a day after the procedure and patients spend on average 3.5 days in hospital.

Following VATS SLB patients will again be discussed at MDT; if no consensus diagnosis can be reached at that point patients will be followed up for at least 6 months which time they might undergo further diagnostic tests, start specific treatment or be observed as per their treating clinician’s usual practice. All cases in which no consensus diagnosis could be reached following initial TBCLB and VATS SLB will again be reviewed at the end of the follow-up period by a MDT panel.
3 SELECTION OF PATIENTS

The eligibility criteria are the standards used to ensure that only medically appropriate patients are considered for this study. Patients not meeting the criteria should not join the study. For the safety of the patients, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other patients with similar diseases, it is important that no exceptions be made to these criteria for admission to the study.

Participants will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

3.1 PATIENT INCLUSION CRITERIA

- 18 years or older with an interstitial pattern on chest CT scan
- MDT decision to obtain histopathology for diagnosis

3.2 PATIENT EXCLUSION CRITERIA

- Platelet count of < 70 x 10^9 / l
- Abnormal coagulation parameters (INR > 1.4) that cannot be corrected easily
- Current anticoagulant and anti-platelet use that cannot be safely stopped
- Recent vascular event or cardiac arrhythmia (within 6 weeks)
- Severe heart failure (NYHA III-IV)
- Pulmonary arterial hypertension with pulmonary artery pressure > 40 mmHg on echocardiography
- Unable to give informed consent (dementia, significant mental illness)
- Pregnancy

3.3 SCREENING PROCEDURES

Written informed consent to enter into the trial must be obtained from participants after explanation of the aims, methods, benefits and potential hazards of the trial and BEFORE any trial-specific procedures are performed. It must be made completely and unambiguously clear that the participant is free to refuse to participate in all or any aspect of the trial, at any time and for any reason, without incurring any penalty or affecting their treatment. Signed consent forms must be kept by the investigator and documented in the CRF and a copy given to the participant.

3.4 CO-ENROLMENT GUIDELINES

Co-enrolment in previous or future trials is permitted. Specifically surplus lung biopsy material will be used in laboratory based studies in “An investigation into the mechanisms of lung injury and
repair in inflammatory, infective, fibrotic and destructive lung diseases including those associated with autoimmune rheumatic diseases.” REC reference: 13/LO/0900
4 SAFETY REPORTING

The principles of GCP require that both investigators and Sponsors follow specific procedures when notifying and reporting adverse events or reactions in clinical trials.

4.1 DEFINITIONS

The definitions of the EU Directive 2001/20/EC Article 2 based on the principles of GCP apply to this trial protocol.

Table 1: Definitions

<table>
<thead>
<tr>
<th>TERM</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Adverse Event (AE)</td>
<td>Any untoward medical occurrence in a patient or clinical trial subject to whom a medicinal product has been administered including occurrences that are not necessarily caused by or related to that product.</td>
</tr>
<tr>
<td>Adverse Reaction (AR)</td>
<td>Any untoward and unintended response to an investigational medicinal product related to any dose administered.</td>
</tr>
<tr>
<td>Unexpected Adverse Reaction (UAR)</td>
<td>An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SPC) or Investigator Brochure (IB) for that product.</td>
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</table>

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)

Respectively any adverse event, adverse reaction or unexpected adverse reaction that:
- Results in death
- Is life-threatening*
- Requires hospitalisation or prolongation of existing hospitalisation**
- Results in persistent or significant disability or incapacity
- Consists of a congenital anomaly or birth defect
- Is another important medical condition***

*The term life-threatening in the definition of a serious event refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that hypothetically might cause death if it were more severe, for example, a silent myocardial infarction.

**Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, that has not worsened or for an elective procedure do not constitute an SAE.

***Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. The following should also be considered serious: important AEs or ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above; for example, a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not result in hospitalisation or development of drug dependency.
4.1.1 ADVERSE EVENTS
Adverse Events include:
- An exacerbation of a pre-existing illness
- An increase in frequency or intensity of a pre-existing episodic event or condition
- A condition (even though it may have been present prior to the start of the trial) detected after trial drug administration
- Continuous persistent disease or a symptom present at baseline that worsens following administration of the study treatment

4.1.2 EXEMPTED ADVERSE EVENTS
Adverse Events do not include:
- Medical or surgical procedures; the condition that leads to the procedure is the adverse event
- Pre-existing disease or a condition present before treatment that does not worsen
- Hospitalisations where no untoward or unintended response has occurred, eg, elective cosmetic surgery, social admissions
- Overdose of medication without signs or symptoms

4.2 INVESTIGATOR RESPONSIBILITIES
All non-serious AEs and ARs, whether expected or not, should be recorded in the patient’s medical notes and details sent to the coordinating centre within 30 days. SAEs and SARs should be notified to the coordinating centre within 24 hours of the investigator becoming aware of the event.

4.2.1.A Seriousness
When an AE or AR occurs, the investigator responsible for the care of the patient must first assess whether or not the event is serious using the definition given in Table 1. If the event is serious and not only related to disease progression, then an SAE Form must be completed and the coordinating centre notified within 24 hours.

4.2.1.B Causality
The investigator must assess the causality of all serious events or reactions in relation to the trial therapy using the definitions in Table 2. There are five categories: unrelated, unlikely, possible, probable, and definitely related. If the causality assessment is unrelated or unlikely to be related, the event is classified as an SAE. If the causality is assessed as possible, probable or definitely related, then the event is classified as an SAR.

Table 2: Assigning Type of SAE Through Causality

<table>
<thead>
<tr>
<th>RELATIONSHIP</th>
<th>DESCRIPTION</th>
<th>SAE TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrelated</td>
<td>There is no evidence of any causal relationship</td>
<td>Unrelated SAE</td>
</tr>
<tr>
<td>Unlikely</td>
<td>There is little evidence to suggest that there is a causal relationship</td>
<td>Unrelated SAE</td>
</tr>
<tr>
<td></td>
<td>(for example, the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (for example, the patient’s clinical condition, other concomitant treatment).</td>
<td></td>
</tr>
</tbody>
</table>

Page 24
### Possible
There is some evidence to suggest a causal relationship (for example, because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (for example, the patient’s clinical condition, other concomitant treatments).

<table>
<thead>
<tr>
<th>SAR</th>
</tr>
</thead>
</table>

### Probable
There is evidence to suggest a causal relationship and the influence of other factors is unlikely.

<table>
<thead>
<tr>
<th>SAR</th>
</tr>
</thead>
</table>

### Definitely
There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

<table>
<thead>
<tr>
<th>SAR</th>
</tr>
</thead>
</table>

#### 4.2.1.C Notification
The coordinating should be notified of all SAEs within 24 hours of the investigator becoming aware of the event.

#### 4.2.2 Notification Procedure

1. The SAE Form must be completed by the investigator (the consultant named on the Signature List and Delegation of Responsibilities Log who is responsible for the patient’s care). In the absence of the responsible investigator, the form should be completed and signed by a member of the site trial team and emailed as appropriate. The responsible investigator should subsequently check the SAE Form, make changes as appropriate, sign and then re-email to the coordinating centre as soon as possible. The initial report must be followed by detailed, written reports as appropriate.

The minimum criteria required for reporting an SAE are the trial number and date of birth, name of investigator reporting, the event, and why it is considered serious.

2. The SAE Form must be emailed to the coordinating centre to Med.lungcool@ucl.ac.uk

3. Follow-up: patients must be followed up until clinical recovery is complete or until the event has stabilised. A further SAE Form, indicated as ‘Follow-up’ should be completed and emailed to the coordinating centre as information becomes available. The patient must be identified by trial number, date of birth and initials only. The patient’s name should not be used on any correspondence and should be deleted from any test results.

#### SAE Reporting
Within 24 hours of becoming aware of an SAE, please email a completed SAE form to the coordinating centre on: Med.lungcool@ucl.ac.uk
4.3 SPONSOR RESPONSIBILITIES

Medically-qualified staff at the coordinating centre and/or the Chief Investigator (or a medically-qualified delegate) will review all SAE reports received. The causality assessment given by the local investigator at the hospital cannot be overruled; in the case of disagreement, both opinions will be provided in any subsequent reports.

The coordinating centre is undertaking the duties of trial Sponsor and is responsible for the reporting of SUSARs and other SARs to the research ethics committees, as appropriate. Fatal and life-threatening SUSARs must be reported to the competent authorities within 7 days of the coordinating centre becoming aware of the event; other SUSARs must be reported within 15 days.

The coordinating centre will also keep all investigators informed of any safety issues that arise during the course of the trial.

The Sponsor will submit Annual Safety Reports in the form of a Developmental Safety Update Report (DSUR) to Competent Authorities (Regulatory Authority and Ethics Committee).
5 DATA COLLECTION AND CONFIDENTIALITY

5.1 DATA TO BE COLLECTED

- Demographic data (Initials, gender, DOB)
- Lung function testing (FEV1, FVC and TLCO)
- Initial clinical differential diagnosis
- Initial HRCT differential diagnosis
- Initial MDT differential diagnosis
- Date, site and operators of TBCLB
- TBCLB procedure length
- TBCLB total radiation dose
- Number of TBCLB biopsies taken
- Site of TBCLB biopsies
- TBCLB complication rates (pneumothorax, moderate or severe bleeding; exacerbations; anesthetic complications)
- Visual pain scale score pre and post TBCLB
- TBCLB size and quality: biopsy size and volume, alveolar tissue present
- EBUS or BAL performed- yes/ no; results
- TBCLB length of stay- discharged home or admitted due to complications
- TBCLB pathological differential diagnosis
- MDT differential diagnosis post TBCLB; consensus diagnosis Y/N; confidence in diagnosis
- VATS SLB Y/N
- Date, site and operator of VATS
- Lobe sampled
- Number of biopsies taken
- Procedure length
- VATS SLB size and quality
- VATS SLB complications (persistent air leak, bleeding, delayed wound healing, exacerbations etc)
- Mortality (if any)- up to 90 days post procedure
- VATS SLB length of stay
- Visual pain scale score pre and post VATS SLB
- VATS SLB pathology differential diagnosis
- MDT differential diagnosis post VATS SLB; consensus diagnosis Y/N; confidence in diagnosis
- 6 month follow up diagnosis if no consensus diagnosis reached
- Interobserver agreement on diagnosis between clinicians; radiologists; histopathologists
5.2 DATA HANDLING AND RECORD KEEPING

The above anonymised data will be entered into a specifically designed database. The database will be password protected and kept on a secure computer at the host institution. Data entry will be performed by a clinical trials practitioner (independent to the investigators) with double entry on 20% of the patients.

5.2.1 DIRECT ACCESS TO PATIENT RECORDS

Access to the data will be restricted to appropriate trial personnel for the purposes of the research and analyses of results only.

Participating investigators should agree to allow trial-related monitoring, including audits, ethics committee review and regulatory inspections by providing direct access to source data and documents as required.

5.2.2 CONFIDENTIALITY

Patient name and address details will be included in the information obtained, but will be kept separate from the medical details. A unique identification number will link the name to the medical details.

The trial personnel, UCL and any regulatory bodies will keep data confidential. Patient names will not be used in any reports about the study and all data is stored in accordance with the Data Protection Act 1998.
6 STATISTICAL CONSIDERATIONS

6.1 OUTCOME MEASURES

The primary outcome measures are the proportion of VATS surgical lung biopsies prevented and cost savings. Secondary endpoints are complication rate, length of hospital stay, sensitivity and diagnostic accuracy of TBCLB.

6.2 SAMPLE SIZE

The mean cost for VATS SLB to the NHS is approximately equivalent to the mean spell cost of an intermediate thoracic procedure with a co-morbidity and complication score of 3-5 which is £3,597 with a lower quartile cost of £2,793 and an upper quartile cost of £4,804 according to the national casemix office. A proportion of patients may experience complications and incur additional costs due to prolonged hospital stay and further intervention. An intermediate thoracic procedure with a co-morbidity and complication score of >6 has an average cost of £4,776, a lower quartile unit cost of £3,624 and an upper quartile unit cost of £5,479(46). There is also variation in clinical practice regarding whether the operating surgeon reviews his patient as an outpatient before the operation. All patients are at least seen once as an outpatient after surgery the cost of which is not included in the procedure tariff. A first patient outpatient appointment is usually costed at £184 and subsequent appointments £102-£143.

Taking into account the variability of co-morbidities, complications and frequency of outpatient appointments a standard deviation has been estimated at £2100. The standard deviation for TBCLB and VATS in 60% of cases is calculated at £1985. Please see appendix A for TBCLB and VATS costing calculations.

A mean difference of £1000 in cost on average would be considered to be acceptable if TBCLB significantly reduced the number of VATS. A total of 66 patients will be required to detect a mean difference of £1000 in cost associated with bronchoscopy, assuming an 80% power and 2.5% significance level.

Assuming a VATS cost of £4125 and a TBCLB cost of £1780 we can expect a saving of £2345. As long as TBCLB reduces the following need for VATS by at least 40% this pathway will be either equal in cost or cheaper than VATS pathway for all.

We expect the number of VATS being reduced by 60%. Under this assumption this sample size is also sufficient to give the study adequate power to assess with z-test at one-sided significance level of 2.5% whether the proportion of patients undergoing VATS SLB is reduced by at least 40%, assuming 80% power. The sample size has been calculated using the statistical software OpenEpi. For further details please see appendix B.

6.3 RECRUITMENT PERIOD

Three to four patients per month are expected to be eligible for the study. At least 90% of these patients will be recruited. Therefore 66 patients should be recruited within two years. The trial is scheduled to open in September 2015.

Comment [TM1]: Dear Gemma- this might change slightly now that we have decided to make it single centre- might go down by 10 patients- in discussion with our statistician
6.4 ANALYSIS PLAN

Demographic and clinical characteristics of the study population will be summarised using mean, standard deviation, median and interquartile range, or counts and percentages, depending on their type and distribution.

The one sample z test at one-sided significance level of 2.5% will be used to determine if there is a reduction of more than 40% in VATS SLB due to TBCLB.

The one sample t-test at two-sided significance level of 5% will be used to investigate whether the mean cost of TBCLB significantly differs from £4,125 (which is the cost associated with VATS). We do not anticipate the distribution of cost to be too skewed. In case of strongly skewed distribution, Wilcoxon rank sum test will be used instead of t-test.

A bootstrapping method will be used to apply for uncertainties in the cost calculation and to validate the result of the cost comparison.

Test accuracy of the TBCLB diagnosis will be estimated calculating sensitivity and negative predictive value (NPV) with 95% binomial confidence intervals.

The length of hospital stay will be summarised using median and interquartile range.

Results from this study will be reported according to the Standard of Reporting Diagnostic Accuracy Guidelines.
7 Ancillary Studies

Surplus lung biopsy material will be used in laboratory based studies in “An investigation into the mechanisms of lung injury and repair in inflammatory, infective, fibrotic and destructive lung diseases including those associated with autoimmune rheumatic diseases.”
REC reference: 13/LO/0900
8 REGULATORY & ETHICAL ISSUES

8.1 COMPLIANCE

8.1.1 REGULATORY COMPLIANCE
The trial complies with the principles of the Declaration of Helsinki: 2013, Fortaleza, Brazil.

It will also be conducted in compliance with the approved protocol, the principles of Good Clinical Practice (GCP) as laid down by the Commission Directive 2005/28/EC with the implementation in national legislation in the UK by Statutory Instrument 2004/1031 (The Medicines for Human Use [Clinical Trials] Regulations 2004) and subsequent amendments, the UK Data Protection Act (DPA number: Z5886415), and the National Health Service (NHS) Research Governance Framework for Health and Social Care (RGF).

8.1.2 DATA COLLECTION & RETENTION
CRFs, clinical notes and administrative documentation will be kept in a secure location (for example, locked filing cabinets in a room with restricted access) and held for 15 years after the end of the trial. During this period, all data should be accessible to the competent or equivalent authorities, the Sponsor, (and other relevant parties?) with suitable notice. The data may be subject to an audit by the competent authorities.

8.2 ETHICAL CONDUCT OF THE STUDY

8.2.1 ETHICAL APPROVALS
Ethical approval for this study will be acquired from the local ethics committee using the Integrated Research Application System and UCLH Hospitals NHS Trust. Approval will be sought. Any further amendments will be submitted and approved by the ethics committee.

All patients will give informed consent sheets (see appendix for information sheet and informed consent form). Patients unable to give informed consent will not be recruited. Patients can withdraw or discontinue the study at any point.

The rights of the participant to refuse to participate in the trial without giving a reason must be respected. After the participant has entered into the trial, the clinician must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the participant. The reason for doing so, however, should be recorded; the participant will remain within the trial for the purpose of follow-up and for data analysis by the treatment option to which they have been allocated. Similarly, the participant must remain free to change their mind at any time about the protocol treatment and trial follow-up without giving a reason and without prejudicing his/her further treatment.
8.3 COMPETENT AUTHORITY APPROVALS

This is not a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC. Therefore, a CTA is not required in the UK.

8.4 OTHER APPROVALS

The protocol will be submitted by those delegated to do so to the relevant R&D department.

8.5 TRIAL CLOSURE

The trial will close when all patients have completed follow-up.
9 INDEMNITY

The management of the research will be covered by UCL insurance for negligent harm. University College London holds insurance against claims from participants for injury caused by their participation in this clinical study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, if this clinical study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical study. University College London does not accept liability for any breach in the hospital’s duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Hospitals selected to participate in this clinical study must provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary can be provided on request.

UCL provides cover for negligence harm arising from the design of the research and NHS indemnity scheme or professional indemnity will apply for negligence harm arising from the conduct of the research (participants recruited at NHS sites only).
10 FINANCE

This trial will be financed through a grant provided by Breathing Matters, and a clinical fellow grant by GSK. A funding decision from Dunhill Medical Trust is awaited.
11 OVERSIGHT & TRIAL COMMITTEES

There are a number of committees involved with the oversight of the trial. These committees are detailed below, and the relationship between them expressed in the figure.

11.1 TRIAL MANAGEMENT GROUP (TMG)

A Trial Management Group (TMG) will be formed comprising the Chief Investigator, other lead investigators (clinical and non-clinical) and members of the coordinating centre. The TMG will be responsible for the day-to-day running and management of the trial. It will meet approximately three times a year at least one of which will be in-person.

11.2 TRIAL STEERING COMMITTEE (TSC)

The Trial Steering Committee (TSC) will be formed of all members of the TMG plus independent members. It will be chaired by Dr Toby Hillman and will have a patient representative, Mr Roger Hacker. The role of the TSC is to provide overall supervision for the trial and provide advice through its independent Chair. The ultimate decision for the continuation of the trial lies with the TSC.

11.3 INDEPENDENT DATA MONITORING COMMITTEE (IDMC)

An Independent Data Monitoring Committee (IDMC) will be formed consisting of two experienced clinicians and one statistician. Reports to the IDMC will be produced by the coordinating centre statistician. The IDMC will meet within 6 months of the trial opening. The IDMC can recommend premature closure or reporting of the trial.
12 PUBLICATION

We intend to disseminate findings from the research in peer-reviewed journals. Clinicians and researchers involved in the project will be acknowledged in written papers.
13 REFERENCES

36. Helena Azcuna MJPI, MD; Myriam Aburto, PhD; Inmaculada Barredo, MD; Jose Javier Echeverria, MD; Luis Tena, MD; Sandra Dorado, MD; Amaia Garcia Loizaga, MD; Arantza Romani, RN; Amaia Aramburu, MD; Ane Uranga, MD; Cristobal Esteban, MD; Alberto Capelastegui, PhD. Cryobiopsy in the Diagnosis of Interstitial Lung Disease. Chest. 2014;145((3_MeetingAbstracts)):254A.


43. correspondence BCAD-p. 2012.


# Appendix A Costing for Transbronchial cryo lung biopsy and VATS SLB

Cost of TBCLB to the NHS assuming 20 cases per year. Cryoprobe equipment is assumed to be only used for TBCLB. In practice a significant number of interventional cases per year are likely to be performed depending on the remit of the interventional bronchoscopy centre therefore capital cost would be reduced and is likely to be overestimated here.

Assumed nursing time per procedure 2h. All staff cost include London weighting.

<table>
<thead>
<tr>
<th>Resource</th>
<th>Cost per year (£) for 20 cases</th>
<th>Cost Per procedure (£)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capital cost of 1 interventional scope (Olympus 1 T)</td>
<td>430</td>
<td>21.5</td>
<td>Capital cost including VAT £30056; spread over 7 years; 4293.7/y – 10% of use for TBCLB</td>
</tr>
<tr>
<td>ERBE Cryo unit – Cryo 2 (newest model)</td>
<td>1372.17</td>
<td>68.6</td>
<td>Capital cost 9605.19 spread over 7 years</td>
</tr>
<tr>
<td>ERBE Cryoprobe</td>
<td>570</td>
<td>28.5</td>
<td>Capital cost 2856 per probe. Can be used for 100 procedures</td>
</tr>
<tr>
<td>Bronchoflex ET tube</td>
<td>1180</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Fogarthy Balloon</td>
<td>500</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>2 consultants (1 respiratory 1 anaesthetics) for 0.5 sessions per week</td>
<td>10757</td>
<td>537.85</td>
<td>Consultant scale YM7201 Financial year 2016/17</td>
</tr>
<tr>
<td>1 ODA Band 5 per session</td>
<td>730</td>
<td>37</td>
<td>£36 151/year</td>
</tr>
<tr>
<td>1 radiographer Band 7 per session</td>
<td>1030</td>
<td>52</td>
<td>£51209/year</td>
</tr>
<tr>
<td>2 nurses, 1 health care assistant, 1 recovery nurse</td>
<td>3010</td>
<td>150</td>
<td>Band 5 £ 36151/year Band 6 £43555/year x 2 HCA £26337/year</td>
</tr>
<tr>
<td>1 CXR per patient</td>
<td>500</td>
<td>25</td>
<td>Tariff cost</td>
</tr>
<tr>
<td>Drug cost</td>
<td>421.8</td>
<td>21.09</td>
<td>1% propofol 50ml £10.1 2ml midazolam £0.45 1g tranexamic acid £3.0 Adrenaline £7.54 Source: BNF</td>
</tr>
<tr>
<td>Sterilisation</td>
<td>1200</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Pathology</td>
<td>2900</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>Administration</td>
<td>1000</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Costs for complications- assuming 10% pneumothorax rate; Length of Stay 2 days</td>
<td>1200</td>
<td>60</td>
<td>£366 Rocket Seldinger drain kit for 5; other kit (tubing/bottle) £30 Bed day cost £225</td>
</tr>
<tr>
<td>Overheads</td>
<td>8840</td>
<td>440</td>
<td>1/3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>35650</td>
<td>1780</td>
<td></td>
</tr>
</tbody>
</table>
## Estimated Cost of VATS SLB to the NHS.

<table>
<thead>
<tr>
<th>Resource</th>
<th>Cost Per procedure (£)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic theatre cost</td>
<td>1598</td>
<td>Includes staff cost, equipment and general consumables but not drugs for 1 hour (source ISD Scotland National statistics based on Golden Jubilee National Hospital)</td>
</tr>
<tr>
<td>Theatre recovery cost</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>2 CXR per patient</td>
<td>50</td>
<td>Tariff cost</td>
</tr>
<tr>
<td>Drug cost</td>
<td>40</td>
<td>Anaesthetic and pain relief post op</td>
</tr>
<tr>
<td>Pharmacy cost</td>
<td>50</td>
<td>TTO cost (medication and admin)</td>
</tr>
<tr>
<td>Sterilisation</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Pathology</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>Administration</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Chest drain</td>
<td>103.2</td>
<td>£366 Rocket Seldinger drain kit for 5, other kit (tubing/bottle) £30</td>
</tr>
<tr>
<td>In-patient stay</td>
<td>787.5</td>
<td>Median 3.5 days at £225/day</td>
</tr>
<tr>
<td>Out-patient appointment</td>
<td>100</td>
<td>1 additional OP appointment with surgeon</td>
</tr>
<tr>
<td>District nurse</td>
<td>60</td>
<td>2 district nurse visits for wound dressing</td>
</tr>
<tr>
<td>Overheads</td>
<td>1031</td>
<td>1/3</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>4125</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Appendix B  Sample Size Calculations

1. **Standard deviation for cost of TBCLB**

**Costs per diagnostic pathway:**

- TBCLB alone without complications: £1702
- TBCLB alone with pneumothorax and presumed LOS 2 days: £2500
- TBCLB without complications followed by VATS SLB (Costed as £4125): £5872
Assuming 20 procedures per year with a VATS reduction of 40% this would result in the following case mix:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number</th>
<th>Cost/procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBCLB</td>
<td>6</td>
<td>1702</td>
</tr>
<tr>
<td>TBCLB + pneumothorax</td>
<td>2</td>
<td>2500</td>
</tr>
<tr>
<td>TBCLB + VATS (intermediate)</td>
<td>12</td>
<td>5827</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>4256</td>
</tr>
</tbody>
</table>

Standard deviation of above scenario is estimated as £1,985

2. Standard deviation for cost of VATS SLB

We have estimated the true cost of a VATS SLB to the NHS in the above table assuming a 1/3 overhead as part of increased costs in a London setting with the same mark-up applied to TBCLB costing. No published data is available of the true cost for VATS SLB. However the National Schedule of Reference Costs (2013-14) for elective inpatients published by the national casemix office quotes the following costs for intermediate thoracic procedures (which includes a variety of thoracic procedures and not only VATS SLB) with varying co-morbidity and complications scores (CC score):

<table>
<thead>
<tr>
<th>Intermediate Thoracic Procedure with CC score</th>
<th>National Average Unit Cost</th>
<th>Lower Quartile Unit Cost</th>
<th>Upper Quartile Unit Cost</th>
<th>Average Bed Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>£2,995</td>
<td>£2,211</td>
<td>£3,935</td>
<td>2.4</td>
</tr>
<tr>
<td>3-5</td>
<td>£3,597</td>
<td>£2,793</td>
<td>£4,804</td>
<td>3.4</td>
</tr>
<tr>
<td>&gt;6</td>
<td>£4,776</td>
<td>£3,624</td>
<td>£5,479</td>
<td>5.5</td>
</tr>
</tbody>
</table>

An intermediate thoracic procedure with a CC score 3-5 most closely correlates with our VATS cost estimation and the average length of stay recorded in the literature. A proportion of patients may experience complications and incur additional costs due to prolonged hospital stay and further intervention. There is also variation in clinical practice regarding whether the operating surgeon reviews his patient as an outpatient before the operation. All patients are at least seen once as an outpatient after surgery the cost of which is not included in the procedure tariff. A first patient outpatient appointment is usually costed at £184 and subsequent appointments £102-£143. Taking into account the variability of co-morbidities, complications and frequency of outpatient appointments a wide standard deviation has been estimated at £2100.

3. Sample Size Calculation

Assuming a VATS cost of £4125 and a TBCLB cost of £1780 we can expect a saving of £2345. As long as TBCLB reduces the following need for VATS by at least 40% this pathway will be either equal in cost or cheaper than VATS pathway for all.

Working: 4125/2345= 1.76

1- 1/1.76 + 0.6 = 1.03
## Sample Size For Comparing Two Means

### Input Data

<table>
<thead>
<tr>
<th>Confidence Interval (2-sided)</th>
<th>95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>80%</td>
</tr>
<tr>
<td>Ratio of sample size (Group 2/Group 1)</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
<th>Difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1985</td>
<td></td>
<td>2100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance</td>
<td>3940230</td>
<td></td>
<td>4410000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size of Group 1</td>
<td>66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample size of Group 2</td>
<td>66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sample size</td>
<td>132</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Difference between the means

Results from OpenEpi, Version 3, open source calculator--SSMean

APPENDIX 9 – LUNG COOL ETHICS APPROVAL
Dear Dr Porter

Study title: LUNG COOL trial - CryOextractiOn of Lung tissue for diagnosis of interstitial LUNG diseases

REC reference: 16/LO/0454
Protocol number: 15/0663
IRAS project ID: 182874

Thank you for your letter of 04 April 2016, responding to the Committee’s request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a Sub-Committee of the REC. A list of the Sub-Committee members is attached.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Miss Christie Ord at nrescommittee.london-camdenandkingscross@nhs.net.

Confirmation of ethical opinion

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

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

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

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).


Where a NHS organisation’s role in the study is limited to identifying and referring potential participants to research sites (‘Participant Identification Centre’), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

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

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett ([catherineblewett@nhs.net](mailto:catherineblewett@nhs.net)), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

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

NHS sites

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

Non-NHS sites

The Committee has not yet completed any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Approved documents

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

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
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<tr>
<td>Covering letter on headed paper</td>
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<tr>
<td>Instructions for use of medical device [NICE ERBE Cryoprobes review]</td>
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<td>Letter from funder</td>
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<td>Letter from sponsor</td>
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<td>Letter from statistician [Stats correspondence]</td>
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<td>Other [Insurance Certificate]</td>
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<td>Other [GSK funding letter]</td>
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<td>Other [Cover letter for REC revisions]</td>
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<tr>
<td>Participant consent form [PIC v2]</td>
<td>V2</td>
<td>04 April 2016</td>
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<tr>
<td>Participant information sheet (PIS) [PIS V2]</td>
<td>V2</td>
<td>04 April 2016</td>
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<tr>
<td>REC Application Form [REC_Form_01032016]</td>
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<td>Research protocol or project proposal</td>
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<td>24 February 2016</td>
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<td>Summary CV for Chief Investigator (CI)</td>
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<td>Summary CV for student</td>
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<td>Summary CV for supervisor (student research)</td>
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<tr>
<td>Validated questionnaire [Visual Analog scale]</td>
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<td>Validated questionnaire [KBUILD]</td>
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Statement of compliance

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

After ethical review

Reporting requirements

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

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

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

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

16/LO/0454 Please quote this number on all correspondence

With the Committee’s best wishes for the success of this project.

Yours sincerely

pp

Ms Heidi Chandler
Vice-Chair

A Research Ethics Committee established by the Health Research Authority
Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments

“After ethical review – guidance for researchers” [SL-AR2]

Copy to: Ms Smaragda Agathou, University College London Hospitals NHS Foundation Trust
London - Camden & Kings Cross Research Ethics Committee

Attendance at Sub-Committee of the REC meeting held in correspondence

Committee Members:

<table>
<thead>
<tr>
<th>Name</th>
<th>Profession</th>
<th>Present</th>
<th>Notes</th>
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<tbody>
<tr>
<td>Ms Heidi Chandler</td>
<td>Deputy Research Delivery Manager</td>
<td>Yes</td>
<td>Vice-Chair</td>
</tr>
<tr>
<td>Miss Jessica Hughes</td>
<td>Director, Corporate Public Policy</td>
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Also in attendance:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position (or reason for attending)</th>
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<tr>
<td>Miss Kirstie Penman</td>
<td>REC Assistant</td>
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Patient Information Sheet: Transbronchial cryoscopic lung biopsy

A transbronchial biopsy is a procedure performed during examination of your lungs with a flexible telescope (bronchoscopy) in which a small piece of lung tissue (biopsy) is removed for analysis. Your consultant has recommended a transbronchial biopsy to find out the cause of the problem in your lungs.

Alternatives
An X-ray or scan can show that you have a problem however a biopsy is more likely to find out exactly what is causing the problem.

The procedure
You won’t be able to eat or drink anything for six hours before the procedure, but you can take your regular medication with a small amount of water. You will be admitted to our surgical or endoscopy unit before the procedure. Please attend at the time stated on your letter. Once ready, you will be taken to the procedure room. Your doctor will use local anaesthetic gel and spray to numb your throat. This can taste unpleasant but only lasts for a couple of minutes. Your doctor will also give you a sedative to help you relax and make you go to sleep. Your oxygen levels and heart rate will be monitored during the procedure. A transbronchial biopsy procedure usually takes half an hour to 45 minutes. Your doctor will pass a flexible telescope (bronchoscope) through your mouth and down into your lungs. Your doctor will use the bronchoscope to examine your airways (bronchi), and then the cryo-probe tip is passed out of the bronchoscope into smaller bronchi to get biopsies from the outer part of the lung. Your doctor will use the cryo-probe to take samples of lung tissue, using an X-ray machine to determine the location within the lung from which the biopsies are taken. Your doctor may also use small amounts of salty water to obtain other further samples for analysis. You will have a chest X-ray after the procedure. Following the procedure, the samples will be examined to find out the cause of your problem.

What to do about your regular medications
Continue your normal medication unless you are told otherwise.
If you take any blood thinning treatment (warfarin, aspirin, clopidogrel, apixaban, rivaroxiban, dabigatran), please make sure that your consultant is aware of this; the biopsy procedure cannot take place unless specific arrangements have been made.
If you are taking diabetic medication, please make sure that your consultant is aware of this as some treatment may need to be altered, and you will usually need to go first on the list.

It is now our policy to copy all letters to the patient for information and to improve your care.
Your GP has this letter and, if you have questions arising from it, please contact him/her in the first instance.
Possible complications

• Pneumothorax, where air escapes into the space around the lung. Usually a pneumothorax is small and does not cause any problems. If it is large, your doctor may need to insert a tube (chest drain) in the space around the lung to re-inflate the lung. This happens in fewer than 1 in 10 cases.
• Bleeding from a biopsy site which is usually minor and stops on its own. It is normal to cough up some streaks of blood for a day or two after the procedure
• Developing a sore throat, which gets better quickly

This is not a definitive list and symptoms will vary with each patient. Please ask your consultant for more information of your individual risks.

Recovery

Once you are awake enough and able to swallow properly, you will be given a drink. You should be able to go home after you have recovered from the sedative, and have had a chest X-ray (normally about one hour after the procedure).

Remember, you won’t be able to drive home after the procedure, to operate heavy machinery, or to make important decisions and you will need someone to collect you from the hospital.

Once at home, if you have any of the following let your doctor know immediately:
• severe chest pain
• sudden breathlessness
• you coughed up more than a tablespoon of blood

For more information, and if you have any queries about the procedure, speak to your consultant.