Study Protocol

Short Title: FIBRO-SAM

Full Title:

Development of Airway Absorption Sampling Methods for Biomarker Assessment in Probable Idiopathic Pulmonary Fibrosis (IPF) Patients

IRAS project Number

239911

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MAIN SPONSOR:

Imperial College London

FUNDER:

Genentech, South San Francisco, California, USA

STUDY CENTRE:

Patients	Healthy volunteers
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REC reference: 20/LO/0176

Protocol authorised by: (Name)	Role	Date	Signature

Study Coordination Centre:

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Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Research Governance and Integrity at:

Joint Research Compliance Office
Imperial College London and Imperial College Healthcare NHS Trust
2nd Floor, Medical School
Norfolk Place
London, W2 1PG

Tel: 0207 594 1872

Every care was taken in writing this protocol and this has been reviewed by the co-investigators. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the ICH-GCP Guideline originating from the Declaration of Helsinki, 1964 and all amendments, as well as the NHS Research Governance Framework for Health and Social Care. It will be conducted in compliance with the protocol, the General Data Protection Regulation (GDPR) 2018 and other regulatory requirements as appropriate.

GLOSSARY OF ABBREVIATIONS

AE	adverse events
BAL	bronchoalveolar lavage
BS	bronchosorption
CCL	chemokine of cysteine-cysteine type
CRF	case report form
CXC	chemokine of cysteine -X- cysteine type
C3M	collagen degradation markers
CRPM	c-reactive protein degraded by MMP
DLco	diffusion factor for carbon monoxide
EL-M7	MMP-7 generated elastin fragments
FEV1/FVC	forced expiratory volume/forced vital capacity
GDPR	General Data Protection Regulation
KL-6	Krebs von den Lungen 6
IgE	immunoglobulin E
IL	interleukin
IPF	interstitial pulmonary fibrosis
MDT	multi-disciplinary team
MLF	mucosal lining fluid
MMP	matrix metalloprotease
MSD	Meso Scale Discovery
NAC	nasal allergen challenge
NS	nasosorption
SAE	serious adverse event
SAM	synthetic absorptive matrix
SP	surfactant protein
sRAGE	soluble receptor for advanced glycation end products

KEYWORDS

Idiopathic pulmonary fibrosis, biomarkers, mucosal lining fluid, nasosorption, bronchosorption, cytokines, chemokines,

STUDY SUMMARY

TITLE	FIBRO-SAM: Development of Airway Absorption Sampling Methods for Biomarker Assessment in Probable Idiopathic Pulmonary Fibrosis (IPF) Patients
DESIGN	Observational study with opportunistic airway sampling in patients with probable Idiopathic Pulmonary Fibrosis and Sarcoidosis, who are undergoing bronchoscopy as part of their diagnosis and/or monitoring
AIMS	To assess molecules reflecting pulmonary fibrosis using novel upper and lower airway sampling methods of absorption; nasosorption, (upper airway) and bronchosorption (lower airway).
OUTCOME MEASURES	Comparing levels of mediators including periostin, surfactant protein D (SPD), CCL18, CXCL13 in blood, nasal and bronchial airway mucosal lining fluid.
POPULATION	Probable Idiopathic Pulmonary Fibrosis patients (IPF) undergoing bronchoscopy, n = 30 Sarcoidosis patients, n = 15 Healthy volunteers, n = 15
ELIGIBILITY	Adults (aged 40 to 85 years) will need to meet inclusion and exclusion criteria. Patients in this study will be selected at the time of their scheduled clinical bronchoscopy at St Mary's Hospital at Imperial College Healthcare Trust and all Healthy controls will be recruited by approaching carers and partners of recruited patients as well as advertising in hospital OPA, public places and local media
DURATION	Clinical Phase: March 2020 to March 2021 (estimated)

Contents

1 INTRODUCTION

Idiopathic Pulmonary Fibrosis (IPF) is a progressive and incapacitating lung disorder that has a variable clinical course, but is generally fatal within a few years (1, 2). The diagnosis of IPF is based on clinical features, lung function tests showing interstitial disease (decreased transfer of carbon monoxide) and usual interstitial pneumonia (UIP) on high resolution computerised tomography (HRCT) scan of the lung fields (3, 4). As part of their diagnostic work up, most patients with probable IPF require bronchoscopy for taking broncho-alveolar lavage (BAL) samples.

Therapy for IPF is gradually being developed with drugs such as pirfenidone (5) and nintedanib (6) showing modest effects. However, the variable and pleiotropic nature of IPF suggests that combination and personalised therapy will be required (7). In recent years, there have been extensive efforts to measure blood biomarkers in this disease, and a number of blood biomarkers have been described in IPF:

- MMP-7, ICAM-1, VCAM-1, CXCL8/IL-8, S100A12 (8)
- surfactant proteins (SP)-A & D (9, 10)
- periostins (11)
- KL6 (12)
- antibodies to heat shock protein (HSP) 70 (13)
- soluble receptor for advanced glycation end products (sRAGE) (14)
- CCL18 (15), CXCL13 (16)
- collagen biomarkers and protease degradation products (collagen neo-epitopes) (17)
- CA19-9 and CA125

Very recently a 52 gene expression profile in blood has been found to be predictive of outcome in IPF (18).

In IPF there is an urgent need to develop new methods for taking lung samples from a bronchoscope to measure mucosal inflammation. Inflammatory mediators in the mucosal lining fluid (MLF) reflect underlying inflammation, but existing bronchoscopic methods (BAL and biopsy) have significant drawbacks.

Bronchosorption involves passing a special medical sampling device down the operating portal of the bronchoscope. Using bronchosorption to obtain samples and identify molecular signatures of respiratory disease, we hope to achieve new fundamental insights and refine the investigation, diagnosis, stratification and monitoring of IPF. We can discover biomarkers to help assess the effects of existing therapies and identify potential targets for novel treatments.

In addition to BAL samples being taken from the bronchoscope for clinical diagnostic purposes, in this study we shall perform upper airway sampling (nasosorption) and this will be compared with the lower airway sampling of bronchosorption.

This study is being carried out for clinical research purposes, is optional for patients, and is unlikely to benefit the patient personally. This study is unlikely to change the clinical diagnosis of a patient and will not change their drug treatment.

Absorption of Airway Mucosal Lining Fluid with Synthetic Absorptive Matrix (SAM)

Synthetic absorptive matrix (SAM)

SAM strips are like blotting paper and are now widely used to obtain nasal MLF by nasosorption having first been used in 2003 (19, 20). These absorbent materials are comfortable to use and can obtain MLF even from inflamed noses at frequent intervals over extended periods of time. There is minimal protein binding to the SAM strip, and proteins in the fluid can be efficiently eluted.

Nasosorption

After a nasal allergen challenge, we have used the technique of nasosorption to absorb mucosal lining fluid from the nose at frequent intervals. Nasosorption uses a small strip of absorbent material on a handle to soak up fluid from the inside surface of the nostril. Nasosorption is a CE marked device that uses medical grade materials and is produced in a specialised medical device manufacturing company. This is placed inside the nostril and left for 60 seconds, keeping light pressure with a finger to absorb fluid before being removed. This technique is very gentle and minimises rubbing and brushing inside the nostril. The material absorbs the fluid and chemicals produced by the nasal cells that can then be extracted and measured. After nasal allergen challenge (NAC) the eluate from nasosorption contains inflammatory mediators, cytokines and chemokines at high detectable levels, and this has been shown after NAC with grass pollen (21, 22), cat allergen (23) and lipopolysaccharide (24). A recent manuscript has described full gene expression changes after NAC with grass pollen (25) In addition, following experimental human rhinovirus (HRV) challenge in asthma patients, nasosorption and bronchosorption were performed to measure IL-15 (26), IL-25 (27), IL-33 (28) and IL-18 (29). A very complete profile of cytokines and chemokines following HRV challenge in asthma has recently been reported (30) (31), and viral load and cytokines have been measured after RSV bronchiolitis of infancy (32). An extensive validation study has been carried out comparing performance of nasosorption with nasal lavage (33).

Bronchosorption:

The Respiratory Medicine group in Imperial College have developed a bronchosorption device that employs a different proprietary medical grade soft fibrous SAM at the tip. The bronchosoprtion device is now CE-marked and has been used in over 1500 patients and healthy volunteers. Bronchosorption has the advantage of being less invasive and causing less dilution than a broncholveolar lavage (BAL). A straw of SAM or synthetic sponge is attached to a leading plastic wire, and placed down a sheath within the operating portal of a bronchoscope. Under direct bronchoscopic vision the SAM is advanced against the mucosa of a main bronchus or segmental bronchus for up to 30 secs, under direct vision the SAM is then withdrawn up the sheath, and MLF eluted from the detached SAM. The eluted MLF may then be analysed for

levels of inflammatory mediators: such as a panel of chemokines and cytokines using the MSD analyser. High detectable levels of mediators of inflammation can then be measured in the bronchial MLF. Prof Onn Min Kon of the Department of Respiratory Medicine at Saint Mary's Hospital has an extensive experience of succesfully sampling over 1000 patients to collect over 1000 bronchosorption samples. The procedure is well tolerated and up to 40μl of fluid is absorbed on a single occasion. The bronchosorption kit is supplied in sterile packs, and has undergone durability and functional testing, and is supplied steriled by gamma irradiation. In the HRV infection model in patients with allergic asthma and healthy controls, both nasosorption and bronchosorption have been carried out to measure IL-15 (26), IL-25 (27), IL-33 (28) and IL-18 (29). Bronchosoorption has been shown to have considerable promise for measuring biomarkers in asthma, and has potential to be used for personalised medicine and selection of specific biologics (30) (31).

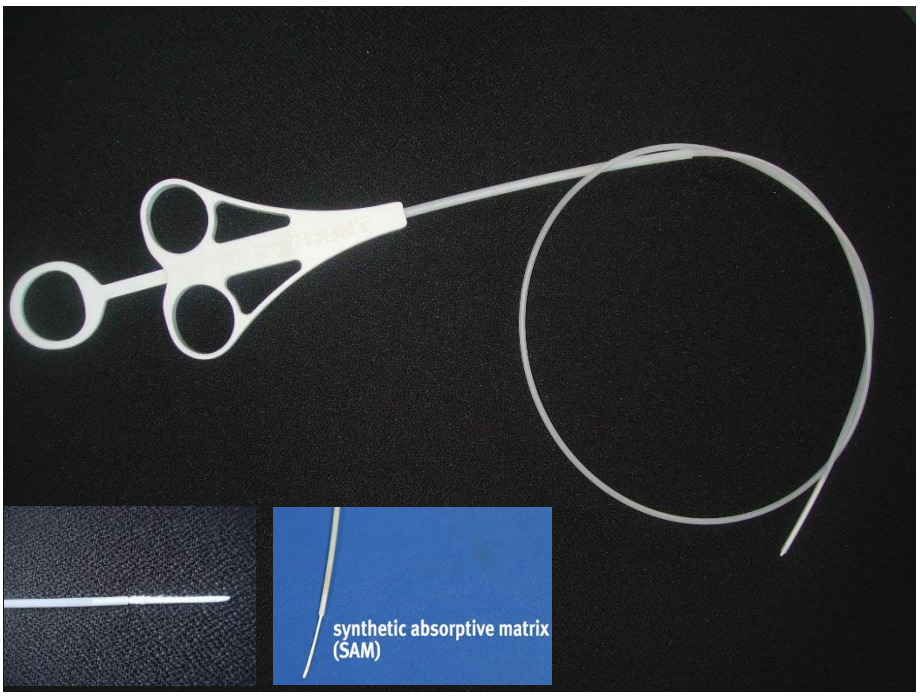

Figure 1: Bronchosorption device

Bronchoalveolar Lavage (BAL):

As part of the bronchoscopy procedure, the doctor intends to collect a BAL sample. This is done by instilling some sterile fluid into the lung and then drawing this fluid back out again with the bronchoscope. The BAL fluid is then studied to help in the diagnosis of lung diseases. For our study, we will collect this fluid for research purposes.

2 STUDY SUMMARY

In patients are having bronchscopy with BAL sampling done for their diagnostic work up, we propose bronchosorption samples be also taken during bronchoscopy for patients with probable IPF (n=30) and sarcoidosis (n=15) who agree to take part in this study.. For our clinical research we shall collect BAL and the following additional samples at the bronchoscopy baseline visit: serum, whole blood, and mucosal lining fluid by absorption: nasosorption, bronchosoprtion and

urine. We will also be collecting serum, whole blood, and nasosorption samples at 3 and 6 month follow-up visits.

In addition we shall study age matched healthy volunteers (n=15) who will not undergo bronchoscopy.From these participants we will collect serum, whole blood, and nasosorption only. Healthy volunteers are included in this study since we need to compare the levels of biomarkers in blood and nasal mucosal lining fluids in patients with probable idiopathic pulmonary fibrosis (IPF) and sarcoidosis with healthy volunteers

We will measure a range of molecules and mediators in the samples collected. We can assess molecules reflecting pulmonary fibrosis in the lungs (BAL), and using novel sampling methods of bronchial mucosal lining fluid (bronchosorption), and also from the upper airway (nasosorption). A critical issue is whether disease in the alveoli and the very small bronchioles will permit molecules to be transmitted by the mucociliary escalator (MCE) up to the larger airways.

Biomarkers in the airways will reflect distal pulmonary fibrosis immunopathology. IPF and sarcoidosis are diseases of fibrosis and granuloma formation and will have different associated biomarkers.

Airway absorption sampling methods and biomarkers can be used to understand molecular mechanisms, diagnose and monitor pulmonary fibrosis. The biomarkers can contribute to personalised medicine through the selection and stratification of patients for specific therapy, to monitor that therapy, and to provide targets for new therapy.

The total study involves a total of 30 subjects with probable IPF, 15 patients with sarcoidosis and 15 healthy volunteers. Recruitment for this study is likely to take between 12 and 24 months. An interim analysis at 12 months will be able to identify any emerging differences in biomarkers between the groups, and allow the nature of biomarkers of inflammation to be adjusted prior to the final analyses. In an exploratory study of novel methods of mucosal sampling in probable IPF and sarcoidosis, we do not know the rate of recruitment, and would prefer not to specify an exact number of patients for when interim analysis is performed.

Biomarkers to be measured:

1. **Blood (serum) and MLF**

There will be a particular focus on 4 biomarkers:
Periostin, surfactant protein D (SPD), CCL18 and CXCL13

A range of other biomarkers will be measured including the following:

- IL-1β, IL-2, IL-8 (CXCL8), IL-12, IL-13, IL-17, IL-23, IL-33
- TGF-β, FGF basic, TNF-β, CTGF
- sICAM-1, sVCAM-1, CRP, SAA
- D-dimer and C3a, C5

Other biomarkers of inflammation may also be measured.

2. **Whole blood for gene expression (mRNA) and DNA sequencing:**

   - To examine in peripheral blood expression of genes and or gene signatures related to IPF and disease progression
   - To examine genetic loci that contribute to IPF risk and disease progression such as muc5b and TERT

3. **Urine**

- Collagen fragments and bioactive lipids involved in inflammation, which may include, but are not limited to C3M, pro-C3, CRPM, and EL-M7, may be measured.

3 STUDY OBJECTIVE

3.1 Research Question and Hypotheses

1. Are levels of periostin, surfactant protein D (SPD), CCL18, CXCL13 and other biomarkers in bronchial mucosal lining fluid (MLF) useful as a biomarker for IPF patients?
2. Do levels of mediators in less invasive upper airway samples (nasosorption) correlate with levels in lower airway samples (bronchosorption)?
3. Do airway levels of these mediators add value when used in conjunction with blood biomarkers of IPF?

3.2 Primary Outcome

Comparison of bronchial lining fluid levels of biomarkers/mediators (periostin, surfactant protein D (SPD), CCL18, CXCL13) in patients with IPF and sarcoidosis.

- Determination of level of significance in the levels of the bronchial mediators between IPF and sarcoidosis patients.

3.3 Secondary Outcomes

1. Comparing mediator levels between airway samples (nasosorption and, bronchosorption) within and across the 3 groups (probable IPF, sarcoidosis and healthy controls). Establish significance between groups and correlations across patient groups for sampling types.
2. Comparing mediator levels and mRNA (gene expression) in blood with airway mediator levels across the 3 patient groups, and assessing the degree of correlation.

3. Comparing mediator levels in airway samples and blood with clinical parameters
4. Comparing urine metabolites across the 3 patient groups.

4 STUDY DESIGN AND SAMPLE COLLECTION

Any patients with probable IPF and sarcoidosis, who will be having bronchoscopy as part of their clinical diagnostic work up will be invited to have extra samples taken, including by the method of bronchosorption. However, the bronchosorption analysis results are not likely to benefit the individual subject.

Patients and healthy volunteers

Potential participants with probable IPF and Sarcoidosis having bronchoscopy as part of their clinical diagnostic work up will be identified by the study doctors and the Multi-Disciplinary Team (MDT) on the basis of inclusion/exclusion criteria. Patients who meet the inclusion and exclusion criteria will then be invited to participate in the study. Health controls will be recruited by approaching carers and partners of recruited patients as well as advertising in clinics, public places as well as local media.

4.1 Consent

Consent to enter the study will be sought after the patient has read and understood and had time to discuss the information contained within the Participant Information Sheet with friends and family. Signed participant consent will be obtained. The right of the participant to refuse to participate without giving reasons will be respected.

After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interests, and the reasons for doing so should be fully documented. In such cases the participants remain in the study for the purposes of follow-up. All participants are free to withdraw at any time from the study without giving reasons and without prejudicing further treatment and care.

4.2 Patient Population

Patients diagnosed with probable IPF and Sarcoidosis will be included. Patients with probable IPF and Sarcoidosis will be selected from clinic lists for patients undergoing bronchoscopy as part of their clinical assessment, and they will be invited to participate in this study that involves additional sampling for clinical research purposes.

Probable IPF patients must have Usual Interstitial Pneumonitis (UIP) on CT scan and will be sub classified by gas transfer (DLco corrected for haemoglobin as detailed below;

- Mild (DLco>60)

- Moderate (DLco 40-60)
- Severe (DLco<40)

Healthy volunteers (age/sex matched, non-smoking, without a clinical history of atopy).

The patient populations will include:

- Probable Idiopathic Pulmonary Fibrosis (IPF), n=30
- Sarcoidosis patients, n=15
- Healthy Volunteers n=15

4.3 Sampling

For patients diagnosed with probable IPF and Sarcoidosis, BAL will be collected on the day of their scheduled bronchoscopy. Blood, nasosorption (NS), and bronchosorption (BS) samples will also be collected. Furthermore, on the day of their optional 3 and 6 months follow up visits, we shall collect blood and nasosorption (NS) samples. ***It is important to stress that bronchosorption samples must be taken before BAL or bronchial biopsy samples***. All patients will have had HRCT of the lung and lung function testing (spirometry and DLco) up to 3 months before bronchoscopy sampling. Healthy volunteers will have a single visit with blood and NS sampling - but no bronchial samples.

With regard to possible coronavirus (COVID-19) infection, this clinical research project will meet Policies and Procedures in conjunction with the NHS, Imperial College London and Public Health England (PHE) guidelines. This will include use of Personal Protective Equipment (PPE) with appropriate clinical facilities and procedures. Appropriate precautions will be taken with sample collection, handling and processing.

5 ENTRY CRITERIA

5.1 Inclusion Criteria for Probable Idiopathic Pulmonary Fibrosis (IPF)

- Adult male or female patients aged 40 to 85 years
- Women of childbearing age should not be pregnant, planning to get pregnant or breast-feeding.
- Command of the English language to be able to give informed consent.
- **Probable IPF** requiring bronchoscopy to confirm the diagnosis, agreed within the local multi-disciplinary team (MDT).,according to the American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/ American Latin Thoracic Association (ATS/ERS/JRS/ALAT) guidelines (2018) (3)
- IPF disease diagnosis within the past 5 years
- Usual Interstitial Pneumonia (UIP) on HRCT scan.
- Recent lung function criteria:
    - Forced vital capacity (FVC) >40% of predicted value.
    - Carbon monoxide diffusing lung capacity (DLco) corrected for haemoglobin >30% of predicted value

5.2 Inclusion criteria for Sarcoidosis

- Adult male or female patients aged 18 years and over
- Women of childbearing age should not be pregnant, planning to get pregnant or breast-feeding.

- Clinical symptoms, CT scan and biopsy diagnosis of sarcoidosis
- Patients with lung parenchymal disease and pulmonary stage II or more
- Recent lung function criteria
    - FVC>50% predicted
    - D_{LCO} >40% predicted

5.3 Inclusion criteria for Healthy Volunteers

- Age between 40 to 85 years, age and sex to match the group with IPF
- Healthy subjects without any diseases that may cause inflammation
- Women of childbearing age should not be pregnant, planning to get pregnant or breast-feeding.
- Currently non-smokers: see exclusion criteria

5.4 Exclusion Criteria

5.4.1 Exclusion Criteria for probable IPF and Sarcoidosis Patients

Respiratory Conditions other than IPF or sarcoidosis:

- Confirmed diagnosis of occupational lung disease
- Drug-induced lung disease or hypersensitivity pneumonitis
- Lung and systemic autoimmune disease including connective tissue disease. Patients with an auto-immune profile considered diagnostic for a specific connective tissue disease will be excluded, even in the absence of systemic symptoms. Non-specific rises in auto antibodies e.g. rheumatoid factor; anti-nuclear antibody etc. will not be used to exclude individuals from the study.
- Asbestosis or other asbestos related disease (pleural plaques, mesothelioma, asbestos pleural effusions)
- Granulomatous lung disease.
- Pulmonary artery hypertension (PAH) requiring a specific treatment.
- Predominant chronic obstructive pulmonary disease (COPD) with forced expiratory volume in 1 second /forced vital capacity (FEV1/FVC) <0.70.
- Patients with active tuberculosis or incompletely treated latent tuberculosis infection
- Lung cancer
- Upper respiratory tract infections in the past 6 weeks.

Systemic Conditions

- History of vasculitis, autoimmune or connective tissue disease
- Known human immunodeficiency virus (HIV) or chronic viral hepatitis
- Clinically significant diseases (other than IPF or sarcoidosis) that may alter respiratory biomarkers: including other respiratory, gastrointestinal, endocrine, haematological, cardiovascular, genitourinary, skin or neurological diseases.
- Recent or ongoing malignant diseases.
- Significant nasal anatomical defects preventing nasal sampling: including hypertrophy of turbinates, major septum deviation, nasal polyposis or recurrent sinusitis and nasal mucosal defects

Bronchoscopy Contraindications

Any contra-indication to bronchoscopy as set out in British Thoracic Society guidelines (34)

Smoking

A detailed smoking history will be taken from all participants: to include total pack years, smoking in the past year, and smoking in the past 2 weeks.
The history will include cigarettes, pipe smoking, cigars, vaping, and shisha.
Any form of smoking is not permitted within 2 weeks of bronchoscopy.

5.4.2 Exclusion Criteria for Healthy Volunteers

- Current inflammatory/ immunological conditions. Any clinically significant diseases that may alter respiratory biomarkers: including respiratory, gastrointestinal, endocrine, haematological, cardiovascular, genitourinary, skin or neurological diseases.
- Recent or ongoing malignant diseases.
- Significant nasal anatomical defects preventing nasal sampling: including hypertrophy of turbinates, major septum deviation, nasal polyposis or recurrent sinusitis and nasal mucosal defects
- Upper respiratory tract infections in the past 6 weeks.
- Cigarette smoking:
  no cigarettes in the last 2 weeks
  not more than 10 cigarettes in the past year
  <10 year lifetime pack history of smoking

5.4.3 Drug Therapy and Concomitant Medication

The following therapy is not allowed for all study population groups:

- Any drugs that may in the opinion of the investigators influence biomarkers of inflammation and fibrosis.
- Pirfenidone, azathioprine, cyclophosphamide, cyclosporine A, oral steroids, Methotrexate, chemotherapy for 3 months
- Treatment with anticoagulant and anti-platelet therapy
- Inhaled steroids for 7 days
- Prescribed anti-inflammatories, salicylates, indomethacin within 7 days

- Over-the-counter (OTC) medications taken within 7 days

5.5 Withdrawal Criteria

Any clinically significant side effects during procedures or withdrawal of consent by the patient /volunteer will necessitate study withdrawal. Patients /Participants will be informed that withdrawal from the study will not affect the care they receive now or in the future and that they are permitted to ask for all previously retained identifiable samples to be destroyed. However, we will inform participants that any samples collected prior to withdrawal will still be used unless they specifically ask for destruction of these samples
All clinical activities will discontinue if there are adverse events related to the sampling procedures performed as part of the study as judged by the, Study Physician and/or the Principal Investigator. In this case participants will be followed up to optimum resolution of the adverse event. If a participant lost capacity to consent during the study, they would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

6 ADVERSE EVENTS

6.1 Definitions

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- Results in death
- Is life-threatening - *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- Requires hospitalisation, or prolongation of existing inpatients' hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Medical judgement should be exercised in deciding whether an AE is serious in all situations. Important AEs 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 outcomes listed in the definition above, should also be considered serious.

6.2 Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

6.2.1 Non Serious AES

All such events, whether expected or not, should be recorded.

6.2.2 Serious AES

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to a pre-existing condition, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs but should be recorded as AEs.

All SAEs should be reported to the Wales RECs 7 Research Ethics Committee where the Chief Investigator considers the event to be:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the Report of Serious Adverse Event (non-CTIMP) Form. The Chief Investigator must also notify the Sponsor of all SAEs.

Contact details for reporting SAEs Fax: 020 331 25751, attention
Please send SAE forms to: Imperial Clinical Respiratory Research Unit (ICRRU)
St, Mary's Hospital
Mint Wing, First Floor, Entrance C
Paddington
London
W2 1NY
Tel: 020 331 25744 (Mon to Fri 09.00 – 17.00)

7 ASSESSMENT AND FOLLOW-UP

7.1 Visits and study procedures for probable IPF and Sarcoidosis patients

Visit 1

The first visit will be on the day of the scheduled bronchoscopy and the following procedures will be carried out:

- **Medical history**:
  Personal details date of birth, next of kin, and gender, ethnicity and contact details will be collected.
- History relating to general health, allergies and drugs (prescription, over the counter and/or herbal medication/ vitamins) will also be collected

- **Smoking History**
  A detailed smoking history will be recorded in terms of pack years, start date, current smoking (past 3 months)

- **Physical and nasal examination**:
  The study doctor will perform a general physical examination including examination of heart and lungs. In addition, the physician will check the nose to make sure that nasal structure is normal and nasal passages are not blocked.

- **Vital signs**:
  Blood pressure, heart rate, respiratory rate and temperature will be checked
  Participant's height and weight will be checked

- **Blood**:
  As part of the bronchoscopy patients will have a cannula (small tube) inserted into a vein in the arm to administer medications such as sedatives. When this has been inserted a sample of blood sample (approximately 25 ml) will be withdrawn from the tube.

- **Urine Sample:**
  Patients will be asked to provide a sample of urine for safety checks and pregnancy testing in women of child bearing age.

- **Nasosorption or nasal mucosal lining fluid absorption:**
  We will use a small strip of absorbent material on a handle to soak up fluid from the inside surface of the nostril (see photograph in Figure 1). This will be placed inside the nostril and left for 60 seconds, keeping light pressure with a finger to absorb fluid before being removed. This technique is very gentle and minimises rubbing and brushing inside the nostril.


Figure 1: Nasosorption

- **Lung function test: Spirometry**
  This is one of the most common lung function tests. It measures how much air the patient can inhale and exhale. It also measures how fast they can empty the air out of their lungs. Spirometry is used to help diagnose breathing problems such as asthma and chronic obstructive pulmonary disease (COPD). This test will only be performed if the doctor feels that it is required

- **Bronchosorption or bronchial lining fluid absorption:**
  As the bronchoscope is passed into the airway, a small strip of absorptive paper is placed on the surface of the airway for up to 1 minute in a similar way to the nasal lining fluid absorption (see photograph in Figure 2). This is a new technique and will be performed by an experienced respiratory physician. The sampling device is a

specially designed medical device that has been produced by an authorised medical device company according to Medical Device Manufacturing Standards.

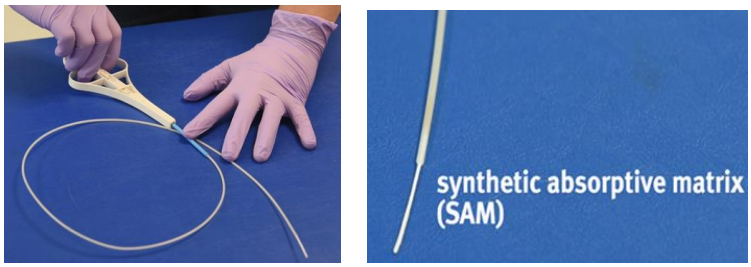

Figure 2: Bronchosorption

- **Bronchoalveolar Lavage (BAL)**:

  During the clinical bronchoscopy, if the doctor intends to collect a bronchial wash sample (BAL), then a small proportion of this fluid will be collected for the study.

Visits 2 and 3

These are follow-up visits, which will take place 3 and 6 months after the bronchoscopy. Both visits will take place at the Imperial College Respiratory Research Unit at St Mary's Hospital, Paddington, London or in the Out -Patient Clinic. Blood, Urine and nasosorption samples will be collected as per visit 1 schedule.

Please see below a summary of study activities

7.1.1 Schedule of assessments for probable IPF and Sarcoidosis patients

	Visit 1 Baseline Bronchoscopy	**Visit 2** 3 months 12-13 weeks post bronchoscopy	**Visit 3** 6 months 24-26 weeks, post bronchoscopy
	at Bronchoscopy suite at St Mary's Hospital	at Out Patients Clinic at St Mary's Hospital or ICRRU	
Lung function tests (including spirometry and DLco)	X	(X) *where clinically indicated*	(X) *where clinically indicated*
Urine	X	X	X
Serum (10 ml) *for Biomarkers*	X	X	X
Whole blood for RNA • 1st visit (10 ml) • 2nd and 3rd visits *miRNA (plasma) and mRNA (gene expression)*	X	X	X
Whole blood for DNA (5 ml)	X		

Upper Respiratory Tract sampling	Nasosorption (2 samples)	X	X	X
Bronchoscopy		X		
Lower Respiratory Tract sampling	Bronchosorption (up to 6samples)	X		
	Bronchoalveolar lavage (BAL)	X		

7.2 Visits and study procedures for healthy volunteers

Participants will be required to attend ICRRU on a single day for up to 3 hours. The visit will involve the following;

- **Medical history**:
- Personal details date of birth, next of kin, and gender, ethnicity and contact details will be collected.
- History relating to general health, allergies and drugs (prescription, over the counter and/or herbal medication/ vitamins) will also be collected

- **Smoking History**
  A detailed smoking history will be recorded in terms of pack years, start date, current smoking (past 3 months)

- **Physical and nasal examination**:
  The study doctor will perform a general physical examination including examination of heart and lungs. In addition, the physician will check the nose to make sure that nasal structure is normal and nasal passages are not blocked.

- **Vital signs**:
  Blood pressure, heart rate, respiratory rate and temperature will be checked
  Participants' height and weight will be checked

The following samples will be collected in the same way as described in section 7 above

- Blood tests
- Urine Sample
- Nasosorption

Please note there will NOT be any bronchoscopy performed on healthy volunteers.

7.2.1 Incidental findings for healthy volunteers

The study physician will discuss any incidental findings with the participant at point of discovery. With the participant's permission these will be communicated to the participant's General Practitioner where necessary. The study Physician will also institute urgent treatment measures as appropriate.

7.2.2 Schedule of assessments for healthy volunteers

Study procedures	Visit 1 at ICRRU, St Mary's Hospital, Paddington, London
	up to 3 hours
Inclusion and exclusion criteria	review
Blood for serum (10ml)	X
Urine	X
Whole blood for RNA (10ml)	X
Whole blood for DNA (5ml)	X
Nasosorption (left and right nostrils)	X

Sample Management

7.3 Sample Storage and Destruction

Samples collected will be stored up to 10 years, or unless the participants specifically requests that the samples be destroyed, or local laws require destruction of the samples. Samples may also be used in future ethically approved studies where the participants have given written consent. However, if samples have been analysed prior to withdrawal, results from the analysis will remain part of the overall research data.
Data arising from sample analysis, including data on germline mutations, will be subject to confidentiality standards.

7.4 Serum

Fill a 10 ml serum separator tube (SST) completely. It is important to thoroughly mix the blood with the clotting activation agent by inverting the tube not less than five times. Allow blood to clot for 30-60 minutes (tube standing upright). Centrifuge at 1500 x g (± 200 x g) for 15 minutes until clot and serum are separated by a well-formed polymer barrier.

Within 20 minutes of the end of centrifugation, use a pipette provided to transfer all serum (upper layer) into an appropriately - labelled 10 ml intermediate tube. Close the cap and invert the tube 10 times to mix the serum.

Use pipette to transfer 1 ml of the serum into each of the four polypropylene tubes labelled "Split 1" through "Split 5".
Freeze all samples immediately at -80°C until the time of shipment on dry ice.

7.5 Whole Blood

7.5.1 Whole blood for DNA

Draw 5 ml blood into 6 ml EDTA tube using standard venepuncture techniques. Mix the blood tube gently by gently inverting tube 5-10 times. Do not shake. Inadequate mixing may result in platelet clumping, clotting and/or incorrect test results. Freeze the sample at -20°C as soon as possible after the draw (must be within 24 hours of draw). Samples should be stored at -80°C until the time of shipment; however, to reduce the risk of tubes cracking, freeze at -20°C first.

7.5.2 Whole blood for RNA

NOTE: You must NOT draw blood directly from the needle into the PAXgeneTM Blood RNA tube. You must NOT use a syringe to draw blood and then add it to the tube.

1. After prepping the venepuncture site with an antiseptic and applying a tourniquet, insert the butterfly needle into the patient's vein.
2. It is important that the PAXgeneTM Blood RNA tubes are at room temperature (18-25° C) prior to use.
3. Fill all required collection tubes, leaving the 2.5 ml PAXgeneTM Blood RNA tubes as the FINAL TUBES to be collected.
4. Hold the PAXgeneTM Blood RNA tube vertically, below the patient's arm to avoid air being transferred into the PAXgeneTM tube or possible backflow from the tube.
5. Ensure that blood has stopped flowing into the PAXgeneTM Blood RNA tube before removing the tube from the holder (at least 10 seconds).
6. Gently invert both PAXgeneTM Blood RNA tubes 8-10 times.
7. Store the PAXgeneTM Blood RNA tubes upright at room temperature (18-25° C) for 2-3 hours before freezing.
8. Freeze at -20°C until shipment.
9. Place a layer of paper towel between the samples and the dry ice.

WARNING: The contents of the PAXgeneTM tube are irritating to the eyes, respiratory system and skin. Refer to the PAXgeneTM Blood RNA Product Circular Section IV (Warnings) in the event of an exposure.

7.6 Airway SAM samples

1. Insert each bronchial SAM into a 2 mL micro-centrifuge tube, along with 300µl of Millipore buffer.
2. Vortex mix the sample for 30 s to wash the SAM of loosely attached fluids and biomolecules.
3. To ensure full sample recovery, perform centrifugal elution by adding the moist SAM to a spin filter mini-column that inserts into the same 2 mL micro-centrifuge tube used for washing.
4. Use sterilized forceps to transfer the moist SAM to the spin filter. Change forceps between samples to prevent contamination.
5. Centrifuge samples for 20 min at 16,000 x g in a mini-centrifuge cooled to 4 °C.
6. Aliquot sample eluate as required and freeze at -80 °C

Further details can be found in the manuscript Thwaites et al., 2018 *J. Vis. Exp*. (131) **(35)**.

7.7 BAL samples

BAL samples will be placed immediately on ice following collection. The total volume recovered will be recorded. In the laboratory, BAL samples will be centrifuged at 1200g for 10 minutes. Supernatant will be aliquoted and stored at -80 ºC. Cell pellets from BAL will be resuspended in a total of 500µl RNA later and stored at -80 ºC.

7.8 Urine Sample

1. Collect a clean catch urine sample into collection cup & store at 4°C (2-4hrs max)
2. Transfer urine into a sterile 50mL screw top plastic centrifuge tube
3. Centrifuge the sample at 1,300xg, 20min, room temperature
4. Decant the supernatant into a new conical tube (being careful not to disturb the pellet in the bottom of the tube)
5. Prepare 5 aliquots of 1mL of the supernatant in low protein binding tubes (GNE usually provides these)
6. Freeze aliquots at -80°C

7.9 Laboratory Procedures, Storage and Shipment

Samples will be barcoded and metadata (including patient number, collection date, sample type, SAM/aliquot #) will be captured by laboratory personnel in internal database/spreadsheet.

Mucosal lining fluid samples will be collected and initially stored frozen at -80 °C without elution. Hence all nasosorption and bronchosorption samples will be stored as non-eluted “samples on SAM” at -80°C in SMH. Prior to shipment to the USA, samples may be eluted and aliquoted according to an agreed elution protocol with a specified buffer at SMH. The weight of nasosorption samples will be recorded.

Serum and urine samples will be aliquoted into 1 ml before storing at -80C to avoid repeated freeze/thaw cycles.

Shipment to Genentech/Roche will be on dry ice. Serum (at least 2ml), Nasosorption (2 SAMs), Bronchosorption (4 SAMs) and BAL will be shipped in two batches; once when all baseline samples have been collected for all patients and once after all time-points (3 months and 6 months) have been collected.

8 STATISTICS AND DATA ANALYSIS

8.1 Sample Size

As previously stated, this is an exploratory biomarker study involving measuring biomarkers of fibrosis in airway mucosal samples (nasosorption and bronchosorption) collected from patients with probable IPF and sarcoidosis compared to healthy volunteers. It is not possible to perform a sample size calculation at this stage, since we do not currently know the magnitude of biomarker differences in nasosorption and bronchosorption samples. Having performed this study, it is hoped that on the basis of particular biomarkers in defined

airway mucosal samples, we can provide sample size calculations for future studies of airway biomarkers in IPF.

8.2 Interim Analysis

The total study involves a total of 30 subjects with probable IPF, 15 patients with sarcoidosis and 15 healthy volunteers. Recruitment for this study is likely to take between 12 and 24 months. An interim analysis at 12 months will be able to identify any emerging differences in biomarkers between the groups, and allow the nature of biomarkers of inflammation to be adjusted prior to the final analyses. In an exploratory study of novel methods of mucosal sampling in probable IPF and sarcoidosis, we do not know the rate of recruitment, and would prefer not to specify an exact number of patients for when interim analysis is performed.

8.3 Statistical Analysis

Data and all appropriate documentation will be stored for up to 10 years after the completion of the study, including the follow-up period.

The major data will be levels of mediators: periostin, surfactant protein (SPD), CCL18 and CXCL13 in blood and bronchial lining fluid.

We shall perform a test of distribution of data (Shapiro-Wilks test) to check normality of data to ensure appropriate statistical tests are applied to the data.

We shall make individual x-y plots for mediators displaying levels for each individual subject within the patient groups (Probable IPF and sarcoidosis) and healthy controls

Summary Statistics

- Normal data with graphs with means and standard error of means (SEM)
- Non-normal data with graphs with medians, quartiles and ranges (box-whisker)

Paired or unpaired t-tests/ANOVA (or equivalent non-parametric tests) will be used to compare mediator levels in blood and airway mucosal lining fluid within groups over time or between different groups at a single time point.

Correlations will be performed to compare mediator levels in blood, and upper and lower airway mucosal lining fluid samples and clinical parameters in patient groups and healthy controls.

Other graphical and analyses methods like heat maps, violin plots, clustering, multivariate analyses and ROC curves will be used as appropriate.

A *P* value of <0.05 is considered significant.

9 REGULATORY ISSUES

This study will adhere to the principles outlined in ICH-GCP Guideline originating from the Declaration of Helsinki, 1964 and all amendments as well and the UK Policy Framework for Health and Social Care Research. It will be conducted in compliance with the protocol, the General Data Protection Regulation GDPR (2018), Human Tissue Act (2004) and other regulatory requirements as appropriate. The Chief Investigator has obtained approval from the Health Research Authority and Research Ethics Committee. The Chief Investigator will also require NHS Capacity and Capability Confirmation letter before accepting participants into the study. Samples and clinical data will be shared with Genentech (South San Francisco, California, USA)

9.1 Consent

Consent to enter the study must be sought from each participant only after a full explanation has been given, a Participant Information Sheet offered and enough time allowed for the participant to consider. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interests, and the reasons for doing so should be fully documented. In such cases the participants remain in the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the study without giving reasons and without prejudicing further treatment and care. If a participant lost capacity to consent during the study, they would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

9.2 Confidentiality

Participants' identification data will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the General Data Protection Regulation (GDPR) 2018.

9.3 Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

9.4 Sponsor

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS Trust taking part in this study.

9.5 Funding

Genentech, San Francisco, California, USA is funding this study.

9.6 Audits

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research

10 STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated by the ICRRU unit coordinator and the delegated Clinical Research Fellow with close support from Prof Trevor Hansel of ICRRU at St Mary's Hospital.

10.1 Sample Shipment

Serum, nasosorption samples, bronchosorption samples, and BAL samples will be shipped to Genentech, San Francisco, California, USA at the end of the study. The samples will be shipped in 2 batches; once when all baseline samples have been collected for all patients and again after all time-points (3 months and 6 months) have been collected.

11 PUBLICATION POLICY

Our aim is to ensure the full dissemination of all study results in medical publications of the highest impact and citation factor. Individual subjects will be strictly anonymised.

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