HypErpolarised Xenon Magnetic Resonance PuLmonary ImAging In PatieNts with Long-COVID (EXPLAIN)

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Chief Investigator: Professor Fergus Gleeson[1]

Investigators: Professor Najib Rahman[1]
Dr Andrew Swift[2]
Professor Jim Wild[2]
Professor Ly-Mee Yu[1]
Dr Betty Rahman[1]
Dr James Grist[1]
Professor Allan Lawrie[2]
Dr Roger Thompson[2]
Dr Emily Fraser[3]
Dr Ling-Pei Ho[1]
Dr Helen Davies[4]
Professor Alex Horsley[5]

Affiliations:
1. University of Oxford
2. University of Sheffield
3. Oxford University Hospitals NHS Foundation Trust
4. University Hospital of Wales
5. University of Manchester

Sponsor: University of Oxford
Joint Research Office
1st floor, Boundary Brook House
Churchill Drive
Headington
Oxford OX3 7GB
E-mail: ctrg@admin.ox.ac.uk

Funders: Cohort A: National Consortium of Intelligent Medical Imaging, University of Oxford

Cohorts B & C: National Institute of Health Research (NIHR)

Chief Investigator Signature:

Statistician Signature:

Disclosures

None

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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1. KEY CONTACTS

Chief Investigator	Professor Fergus Gleeson fergus.gleeson@oncology.ox.ac.uk
Sponsor	University of Oxford Joint Research Office, Boundary Brook House Headington, Oxford, OX3 7GB Office: +44 01865 616480
Funder(s)	Cohort A: National Consortium of Intelligent Medical Imaging, University of Oxford Cohorts B & C: National Institute of Health Research (NIHR)
Clinical Trials Unit	Oxford Respiratory Trials Unit
Statistician	Professor Ly-Mee Yu
Committees	Trial Management Group Trial Steering Committee ORTU Safety Oversight Group The Chair of the TMG will be the Chief Investigator of the trial. The Chair of the TSC will be Simon Padley, Royal Brompton Hospital *All of the above committee details are within the group/committee charters.*

2. LAY SUMMARY

We wish to understand why some individuals with Long-COVID struggle with breathlessness on exertion (when active) and have a reduced ability to exercise. To do this, we will use MRI scanning and a special gas (hyperpolarised xenon) which is breathed in during the scan. The xenon gas is harmless in the quantity we use. This technique shows the movement of xenon within the lungs and moving out of the lungs into the bloodstream, similar to how oxygen is absorbed. In patients hospitalised with COVID-19, we found that the xenon MRI scans several months after discharge showed lung damage, even when other tests were normal. Importantly, on follow-up imaging, some have remained abnormal.

We will study more post-hospitalised patients with Long COVID (Cohort A) to determine if our initial findings are confirmed in a larger number of patients. We will also use the same scan technique in individuals with long-COVID not admitted to hospital (Cohort B), who have ongoing breathlessness on exertion to see if they have lung damage. If lung abnormalities are found, we will assess how severe they are, whether they provide an explanation for the ongoing breathing problems, and whether they improve over time. To do this, we will compare scans in individuals with long-COVID who are breathless on exertion (Cohort B, Groups 3 & 4) to those without breathlessness as a symptom (Cohort B, Group 5), and to patients infected by the virus but didn't have any symptoms (Cohort C), enabling us to see if breathing problems are related to lung damage.

We will also give intravenous contrast to look to see if the lung problems are due to blood clots, and also perform a cardiac MRI scan at the same time in some of the participants attending for their Xenon MRI scans. This sequence has already been studied in another research study, and we will learn from this and use it along with our Xenon scans to identify if there is cardiac damage either as the cause of breathlessness or associated with abnormalities seen on the Xenon scans.

Our aim is to further our understanding of some of the factors that cause symptoms in Long-COVID, and provide a much needed explanation to individuals struggling with breathlessness. Learning more about the nature of damage within the lungs through xenon MRI may help with the future development of treatments, and provide a reliable way of measuring the treatment response over time.

3. SYNOPSIS

In this study we aim to identify and characterise the extent of lung and possibly cardiac damage in patients attending Long COVID clinics, whether they were infected in the first wave in the Spring of 2020, or in subsequent waves.

Study Title	HypErpolarised Xenon Magnetic Resonance PuLmonary Imaging in PAtieNts with Long COVID
Short title	EXPLAIN
Study registration	ISRCTN TBC
Sponsor	University of Oxford Joint Research Office 1st floor, Boundary Brook House Churchill Drive Headington Oxford OX3 7GB E-mail: ctrg@admin.ox.ac.uk
Funder	Cohort A: National Consortium of Intelligent Medical Imaging, University of Oxford Cohorts B & C: National Institute of Health Research
Study Design	Prospective observational cohort study
Study Participants	Cohort A: Post-hospitalisation cohort (100) 100 Hospitalised* Long COVID patients, with normal or near normal CT scans Cohort B: Non-hospitalised cohort (250) 200 participants – Non-Hospitalised Long COVID (NHLC) clinic attendees with symptoms of breathlessness 50 participants - Non-Hospitalised Long COVID (NHLC) clinic attendees with no symptoms of breathlessness Cohort C: Controls (50) 50 control participants with a positive COVID test but no symptoms of Long-COVID *Hospitalised refers to participants that required hospital management of COVID-19 infection.
Sample Size	400 participants

Planned Study Period	Planned start date: 01 Nov 2021 Planned end date: 31 Oct 2023 Total project duration: 24 months		
Planned Recruitment period	18 months		
	Objectives	Outcome Measures	Timepoint(s)
Primary	To evaluate, using HPX-pMRI whether unexplained exertional dyspnoea in NHLC is due to lung damage	a) The detection of diffusion and/ or perfusion defects	a) Baseline scan
		b) The determination of whether detected abnormalities correlate with symptoms of breathlessness	b) Baseline and follow-up scan
		c) The degree of pulmonary damage and change detected on follow up scanning	c) End of study
Secondary	To determine if there are cardiac abnormalities associated with HPX-pMRI-detected pulmonary abnormalities or present alone as a cause for NHLC patient breathlessness	The detection of cardiac MRI abnormalities	Baseline scan
Intervention(s)	Not applicable		
Comparator	Not Applicable		

4. ABBREVIATIONS

ACE-2	Angiotensin converting enzymes-2
CI	Chief Investigator
CFS	Clinical Frailty Survey
CMR	Cardiovascular Magnetic Resonance
CoV	Coronavirus
COVID-19	Coronavirus disease 19
CRF	Case Report Form
CT	Computed Tomography
ECG	Electrocardiograph
eGFR	Estimated Glomerular Filtration Rate
EQ5D-5L	EuroQol- 5 Dimension 5 Level descriptive system
FACIT-F	The Functional Assessment of Chronic Illness Therapy – Fatigue Survey
GAD-7	Generalised Anxiety Disorder survey
GCP	Good Clinical Practice
GP	General Practitioner
HPX	Hyperpolarised Xenon
HPX-pMRI	Hyperpolarised Xenon Perfusion Magnetic Resonance Imaging
HRA	Health Research Authority
ICF	Informed Consent Form
IEP	Image Exchange Portal
ILD	Interstitial lung disease
LC	Long COVID
LFT	Lung Function Test
mBORG	Modified BORG Score
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NCIMI	National Consortium of Intelligent Medical Imaging
NHLC	Non-Hospitalised Long COVID
NHS	National Health Service
ORRU	Oxford Radiology Research Unit
ORTU	Oxford Respiratory Trials Unit
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PHQ	Personal Health Questionnaire
RBC:TP	Red Blood Cell/ Tissue Plasma ratio
R&D	NHS Trust R&D Department
REC	Research Ethics Committee

RGEA	Research Governance, Ethics & Assurance, University of Oxford
SARS-CoV	Sudden Acute Respiratory Distress Syndrome Coronavirus
SOP	Standard Operating Procedure
SAE	Serious Adverse Effects
TTE	Transthoracic Echocardiography
Xe	Xenon
6MWT	6-minute walk test

5. BACKGROUND AND RATIONALE

Scientific background

COVID-19 pneumonia has specific features that differ from other respiratory viral infections. Acute COVID-19 pneumonia causes an unusual pattern of lung injury giving rise to early and profound hypoxaemia that is often refractory to oxygen therapy. This clinical picture is suggestive of excessive “shunting” of blood through poorly ventilated regions of the lung from which oxygen cannot be extracted, and of severe ventilation-perfusion mismatch. This may be caused by dysregulation of pulmonary vascular perfusion due to direct damage of the pulmonary microvasculature by the SARS-CoV-2 virus and/or the presence of multiple thrombi. Evidence for this includes radiological reports of abnormal pulmonary vessels and lung histology that demonstrates viral particles within pulmonary endothelium alongside destruction of the vasculature and the presence of microthrombi.

Hospitalised patients with severe COVID-19 pneumonia may develop persisting interstitial abnormalities within the areas of acute abnormality. Where this is the case, exercise limitation and exertional dyspnoea are not surprising. However, nearly all NHLC patients have normal CT scans with no structural cause for their symptoms identifiable. Dual energy CT (DECT) has been reported to identify perfusion defects due to microthrombotic disease in acute COVID-19 infection but is also nearly always normal in NHLC. The Oxford University Hospitals NHS Foundation Trust Radiology department has identified only one positive scan in a local case review of 100 patients with LC. Pulmonary function tests assume homogeneity within the lungs, giving a ‘readout’ for the entire lung, and hence are often “normal” in this population. We hypothesise that an investigation which provides regional data on the function of the alveolar/capillary interface (i.e. anatomical and functional for gas exchange) is required to delineate damage from COVID-19, or to thoroughly exclude the lung as the site of pathology in NHLC.

Proof of principle for our hypothesis is provided by the early published results from the Oxford HPX-MRI study - Post COVID-19 disease follow-up imaging using hyperpOlariSed xenon MRI and CT (C-MORE POST), which has shown that there are specific defects in gaseous diffusion across the alveolar epithelial-capillary membrane in post-hospitalisation Long COVID-19 patients, despite a normal CT scan[45]. These findings imply ongoing impairment in ventilation-perfusion matching and diffusion impairment due to persistent damage of the alveolar-capillary membranes in the lungs during the recovery phase of the illness. This may partially explain the observation that survivors (including never-hospitalised patients) have noted a prolonged recovery characterized by breathlessness, persistent fluctuations in oxygen saturation and functional limitation. Hyperpolarised xenon MRI (HPX-MRI) is a state-of-the art imaging modality that is safe, reproducible and quantitative. It does not involve radiation exposure and is independent of patient respiratory effort. It enables sensitive regional investigation of the lung parenchyma and measures pulmonary ventilation and gas transfer across the alveolar epithelium into the capillaries and red blood cells.

The ability of Xenon to dissolve through the alveolar epithelium into the lung parenchyma and capillary blood may enable detection of changes in Long COVID-19 which is not possible using other imaging

techniques. The HPX-MRI report from Wuhan demonstrated abnormal gas-exchange detected by HPX-MRI Chest in post-hospitalised COVID-19 patients soon after discharge. In dyspnoeic patients following hospitalisation with COVID-19, diffusion abnormalities have been identified at 3-6 month after discharge, and persist in a proportion 6-12 months later (Oxford and Sheffield on-going prior trial data, which is unpublished) including non-ventilated patients. CT scans in these patients are normal or near normal indicating that this modality is not sensitive enough to pick up subtle abnormalities in the lung microstructure and/or pulmonary vasculature. Additionally, 9 Oxford patients have been scanned on two occasions, 3 months apart, and some have not shown significant improvement in the degree of diffusion impairment. We have also performed a few HPX-MRI Chest in NHLC patients as part of our pilot NHLC study, and some of them have shown dissolved phase defects similar to the post-hospitalisation patients.

Long-COVID is a complex and poorly understood syndrome with more than 200 symptoms described by sufferers. It is similar in some respects to Post-Viral Chronic Fatigue Syndrome (CFS), although there is one particularly interesting distinction; 80% of patients report breathlessness as a primary symptom. This is notably absent from prior descriptions of CFS. The mechanisms driving breathlessness and exertional dyspnoea in Long-COVID remain unclear and conventional investigations are typically unremarkable.

The mechanisms underlying the symptoms of Long-COVID are likely to be multiple, overlap and vary between patients. In patients who experience breathlessness at rest or suffer from acute recurrent episodes not related to exertion, a Breathing Pattern Disorder is commonly identified. Physiotherapy can help restore a normal breathing pattern and has been successful in alleviating symptoms in many. There are also a significant subset of Long-COVID sufferers who have reproducible breathlessness on exertion that limits daily activities. In these patients, we question whether exertional dyspnoea may be secondary to persistent abnormalities caused by loss of integrity or low-grade inflammation around the alveolar membrane and/or pulmonary vasculature. Evidence for this includes: 1) The HPX-MRI Chest findings in the post-hospitalised patients as described above, 2) Local data demonstrating that lung function tests, although usually normal, frequently show a gas transfer (a marker of alveolar and pulmonary vascular integrity) disproportionately lower than the accompanying lung volumes, 3) A proportion of patients exhibit evidence of oxygen desaturation on exertion, 4) The recently published C-MORE and CoverScan studies demonstrating MRI anomalies in multiple organs following COVID-19 infection, indicating that the virus can cause long-lasting changes, 5) Acute COVID-19 infection is highly prothrombotic, with up to 30% of those admitted to critical care found to pulmonary embolic disease on CT Pulmonary Angiograms. Furthermore, autopsy specimens have demonstrated the presence of widespread microthrombi within the pulmonary vasculature, and these vascular abnormalities may account for exercise intolerance.

The management of Long-COVID requires a holistic and multidisciplinary approach. However, the proportion of patients who struggle and are functionally incapacitated by breathlessness is significant, and it is frequently breathlessness that limits return to normal activity. As such, there is an urgent and

unmet need to understand the biological factors underpinning dyspnoea and exercise intolerance. Investigations such as HPX-MRI Chest combined with perfusion MRI Chest (HPX-pMRI – this involves injection of gadolinium contrast) provides highly sensitive and integrated information about the structure and function of the lungs and pulmonary vasculature that cannot be obtained through conventional investigations. Identification of abnormalities may potentially 1) impact on therapeutic strategies to potentially limit or improve this complication in those suffering with post-COVID syndrome 2) provide pathophysiology insights of disease and 3) provide information of the prevalence of pulmonary damage and the medium to long term burden of disease post COVID-19 infection.

Currently, patients diagnosed with Long-COVID attending specialist clinics commonly have no identifiable cause for their symptoms. Patients often undergo numerous investigations for exertional breathlessness, including CXRS, CT scans, lung function and echocardiography, which are normal in the vast majority. Over-investigation can heighten anxiety and exacerbate the psychological interpretation of symptoms; a single investigation that may provide detailed granular information about the function and structure of the lungs and pulmonary vasculature would supersede the need for numerous less sensitive tests.

We have shown in post-hospitalised patients that HPX-MRI Chest scans identify abnormalities when all other tests are non-informative. Determining whether diffusion impairments also exist in NHLC patients with exertional breathlessness will advance our understanding of some of the biological mechanisms driving Long-COVID symptomatology. It will help provide an explanation to sufferers frustrated at the lack of current answers and may potentially enable their better longer-term management. Conversely, HPX-pMRI Chest, if normal, will provide reassurance to patients that they have no lasting lung damage and this may also facilitate rehabilitation. HPX-pMRI Chest may potentially provide insight into the nature and trajectory of NHLC abnormalities and might also be used as a method to assess the effects of proposed medical interventions - preliminary discussions have been had with the HEAL-COVID investigators.

All patients recruited will have a low dose CT scan which will be scored as per our Radiology paper; this should be normal or nearly normal, as determined by study investigators, for them to be recruited (patients with post-COVID-19 Interstitial Lung Disease are being recruited into the NIHR funded UKILD-PCF study). Low dose CT has been shown to reduce radiation exposure, enabling imaging to be repeated if required to assess evolving changes, and adequately depicts interstitial lung disease. It is also now possible to differentiate and quantify CT changes, and to subdivide the changes identified. We will be able to co-register the CT and HPX-pMRI Chest scans, as both will be performed at approximately 1 litre inspiration.

Effects of COVID-19 on the heart

The relationship between COVID infection and cardiac disease is also important. Data from at least four major clinical studies of COVID-19 patients from China suggest that evidence of myocardial injury can be detected in 7-20%. Defined by an increase in cardiac biomarkers above the 99th percentile of normal

range or a new abnormality on electrocardiography or echocardiography, cardiac injury was noted to be far more common in patients (up to 46 % of cases) with more severe disease and non-survivors. Conversely, mortality was higher in patients with pre-existing cardiovascular disease or risk factors for coronary disease who contracted COVID-19. These findings suggest that cardiac complications are common and associated with disease severity. A number of mechanisms have been suggested to promote myocardial injury in SARS-CoV-2 infections. These range from a type of fulminant myocarditis, caused by cytokine storm, to infectious viral myocarditis and acute coronary syndrome. In support of a reactive myocarditis, several studies have demonstrated an acute rise in inflammatory cytokine levels and C-reactive protein, which is seen to precede the rise in serum biomarkers of myocardial injury. Experimental rabbit models have also demonstrated that SARS COV-2 can directly infect cardiomyocytes with the help of angiotensin converting enzymes-2 (ACE-2) receptors, raising the possibility of a viral myocarditis. Another potential cause for myocardial injury is an acute coronary syndrome due to plaque rupture. A post-mortem study of 14 patients with SARS revealed that 15.9% of patients display histopathological evidence of myocardial infarction. Cases of a stress induced myocarditis such as Takotsubo cardiomyopathy have also been reported during other respiratory viral infections. Cardiac magnetic resonance (CMR) provides an excellent opportunity to non-invasively interrogate the myocardium for a range of pathological abnormalities. The high sensitivity and specificity of CMR for diagnosing cardiac diseases could help establish the underlying cause of myocardial injury in COVID-19 patients. Right ventricular dysfunction is a frequent finding in patients with COVID-19 both acutely and in months after discharge. Abnormalities in the right heart are believed to reflect increased pressures within the pulmonary circulation. Transthoracic echocardiography (TTE) has the ability to definitively detect an increased right heart pressure and is widely used for the assessment of pulmonary hypertension and its causes (e.g., diastolic dysfunction). It is currently unknown whether right heart pressures or function normalise in patients who recover from COVID-19 or whether this remains abnormal months after the acute illness is overcome. The assessment of both myocardial tissue characteristics on CMR and chamber pressures on TTE could accelerate the development of cardiac-protective pathways and treatment options intended to alleviate myocardial injury during respiratory viral epidemics. From a prognostic point of view, the presence and extent of myocardial fibrosis or scar, as detected on CMR, may be helpful in stratifying risks in COVID-19 patients and could identify those in need of more long-term cardiac support (e.g., those with extensive myocardial injury or patients with asymptomatic pre-existing cardiac diseases).

Cardiac Subset (Oxford Only)

We will perform cardiac MRI in patients recruited to cohort B who will be scanned in Oxford, to determine whether HPX-pMRI Chest abnormalities are associated with cardiac impairment, which may potentially contribute to breathlessness.

Expected clinical value of this research

COVID-19 is a public health emergency of global proportions. We expect that the proposed work will provide important and novel insights into persistent exertional dyspnoea in COVID-19 patients. These findings will aid the development of treatment pathways intended to prevent and limit persistent exertional dyspnoea in these patients and potentially in future viral pandemics. A deeper understanding of the impact of COVID-19 on the quality of life of individuals and potential need for ongoing medical surveillance will also be realised through this study.

6. OBJECTIVES AND OUTCOME MEASURES

	Objectives	Outcome Measures	Timepoint(s)
Primary	To evaluate, using HPX-pMRI whether unexplained exertional dyspnoea in NHLC is due to lung damage	a) The detection of diffusion and or perfusion defects	a) Baseline scan
		b) The determination whether detected abnormalities correlate with symptoms of breathlessness	b) Baseline and follow-up scan
		c) The degree of pulmonary damage and change detected on follow up scanning	c) End of study
Secondary	To determine if there are cardiac abnormalities associated with HPX-pMRI-detected abnormalities or present alone as a cause for NHLC patient breathlessness	The detection of cardiac MRI abnormalities	Baseline scan

7. STUDY DESIGN

This is a multi-centre prospective, observational, cohort study comprising of 3 patient cohorts. The study will recruit patients in Cohort A & B through hospital based Long-COVID clinics and Cohort C will be recruited via advertising. Participants attending Long-COVID clinics with a normal CXR will be identified

by the Clinician and informed of the study. If they agree to be contacted, a member of the research team will contact them, explain the study, and assess eligibility. They will then invite them for a baseline visit.

Cohort C will be recruited using posters placed locally (as per our standard practice for local recruitment), A member of the research team will explain the study and invite them for their visit.

The participants will be invited to undertake their baseline study visit at one of the 4 recruiting centres, Oxford, Sheffield, Cardiff or Manchester. At the baseline visit participants provide consent (unless obtained prior to baseline visit), their demographics, past medical history and smoking history will be collected. If necessary, their renal function will be measured according to local hospital policy. They will have their heart rate, blood pressure and oxygen saturations recorded. They will undertake lung function testing (unless testing has been performed within 6 weeks prior to baseline scan), and either a 1 minute sit-to-stand test, or a 6 minute walk test where appropriate. They will also be asked to complete a set of questionnaires.

A low dose Chest CT scan and HPX-pMRI Chest will be performed either at their baseline visit (unless CT scan has been performed within 6 weeks prior to baseline visit) at Oxford and Sheffield. Sheffield will be responsible for implementing a standardised imaging protocol across the two sites and for the central analysis of the MRI data. Patients from Cardiff and Manchester will therefore travel for the MRI scanning at either Oxford or Sheffield following referral pathways that have been tested in previous studies.. If the participant has already had a low dose Chest CT (as per the British Thoracic Society Guidelines for patients with Long COVID and breathlessness), and this was normal or near normal, it will not be repeated.

Following their baseline visit, all participants will be invited to complete up to two additional follow-up visits depending on the outcome of their baseline results, see appendix B).

If an abnormality (a result outside the normal range) is found on the HPX-pMRI Chest scan at baseline the participant will be invited to have all study assessments repeated at visit 2. If at visit 2 further HPX-pMRI abnormalities are found, another follow-up visit, visit 3, will be offered to the participant to have all study assessments repeated again.

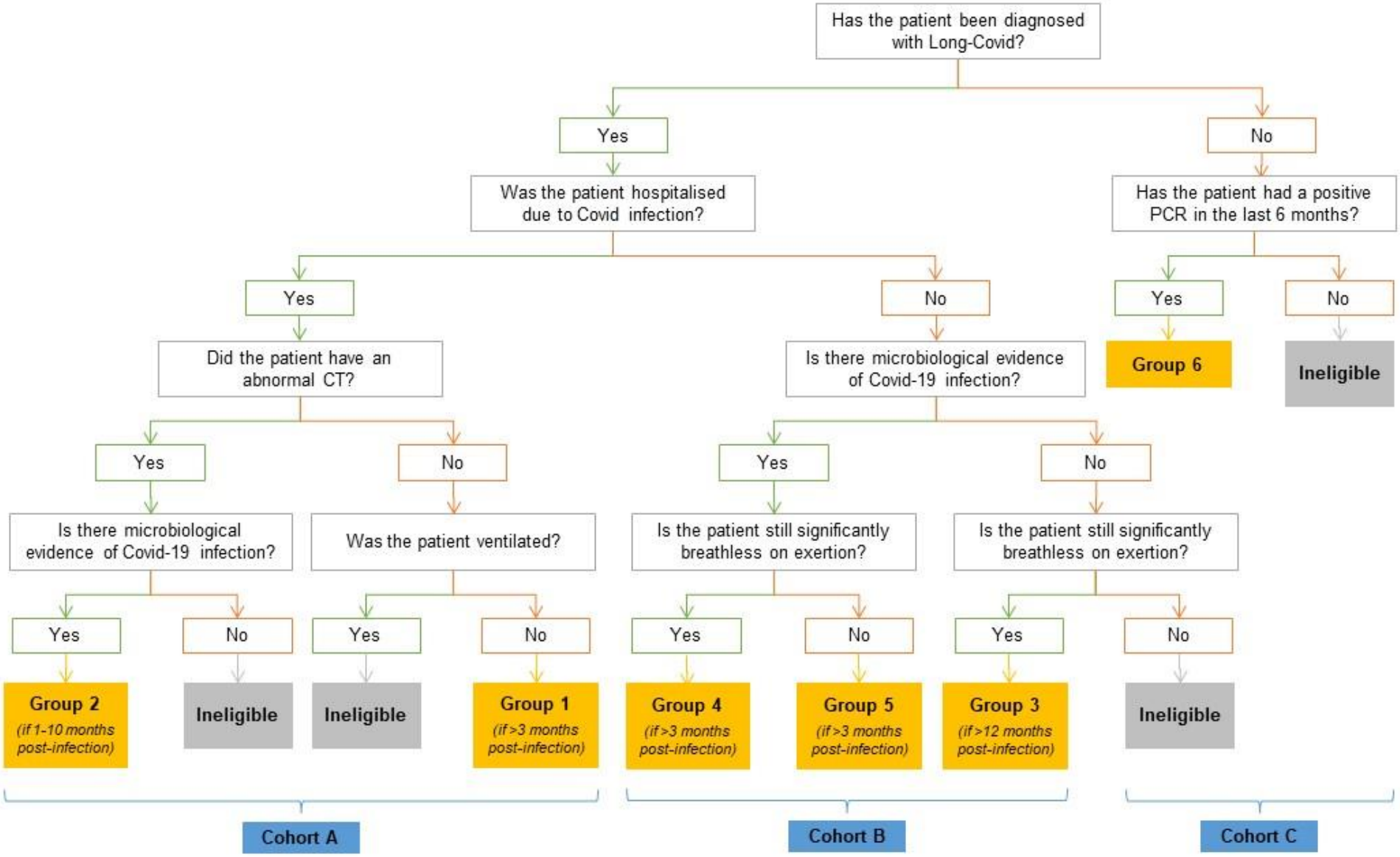
If at the baseline visit no abnormalities are found, the participant will be invited to complete the same set of study questionnaires electronically only 3-12 months post their baseline visit.

All of this information will be recorded on the trial database.

8. PARTICIPANT IDENTIFICATION

8.1. Study Participants

Study participants will be identified to join one of the following cohorts, explained in the diagram below:


Has the patient been diagnosed with Long-Covid?
Yes
No
Was the patient hospitalised due to Covid infection?
Has the patient had a positive PCR in the last 6 months?
Yes
No
Yes
No
Did the patient have an abnormal CT?
Is there microbiological evidence of Covid-19 infection?
Group 6
Ineligible
Yes
No
Yes
No
Is there microbiological evidence of Covid-19 infection?
Was the patient ventilated?
Is the patient still significantly breathless on exertion?
Is the patient still significantly breathless on exertion?
Yes
No
Yes
No
Yes
No
Yes
No
Group 2 (if 1-10 months post-infection)
Ineligible
Ineligible
Group 1 (if >3 months post-infection)
Group 4 (if >3 months post-infection)
Group 5 (if >3 months post-infection)
Group 3 (if >12 months post-infection)
Ineligible
Cohort A
Cohort B
Cohort C

8.2. Inclusion & Exclusion Criteria

Cohort A – Post-hospitalised

Inclusion Criteria

1. Aged 18 years or over
2. Willing and able to give informed consent
3. Previously hospitalised with COVID-19 pneumonia
4. Diagnosis of Long-COVID or 'post-COVID syndrome' (as defined by NICE 2020) made through specialised assessment at a designated long-COVID clinic
5. **Group 1 ONLY:**
    a. More than 3 months post-infection
6. **Group 2 ONLY:**
    a. 1 - 10 months post-infection
    b. Microbiological evidence of COVID-19 infection
    c. Evidence of architectural distortion on CT

Exclusion Criteria

1. Pregnant, lactating or planning pregnancy during the course of the study
2. Known chronic renal impairment, with EGFR below 60 mL/min. – this will be measured according to local hospital policy.
3. Known prior contrast media reaction
4. Inability to lie flat for imaging.
5. Contraindications to MRI examinations as locally determined.
6. Any other reason, as determined by the study investigators, that renders the participant ineligible for the study.
7. COVID infection in the last 3 months
8. **Group 1 ONLY:**
    a. Participant received mechanical ventilation

Cohort B – Non-Hospitalised

Inclusion Criteria

1. Aged 18 years or over
2. Willing and able to give informed consent
3. **Group 3 ONLY:**
    a. More than 12 months since infection
    b. Clinical diagnosis of Long-COVID or 'post-COVID syndrome' (as defined by NICE 2020) made through specialised assessment at a designated Long-COVID clinic
    c. Significantly breathless upon exertion

4. **Group 4 ONLY:**
    a. More than 3 months since infection
    b. Microbiological evidence of COVID-19 infection
    c. Clinical diagnosis of Long-COVID or 'post-COVID syndrome' (as defined by NICE 2020) made through specialised assessment at a designated Long-COVID clinic
    d. Significantly breathless upon exertion
5. **Group 5 ONLY:**
    a. More than 3 months since infection
    b. Microbiological evidence of COVID-19 infection
    c. Clinical diagnosis of Long-COVID or 'post-COVID syndrome' (as defined by NICE 2020) made through specialised assessment at a designated Long-COVID clinic
    d. No respiratory symptoms

Exclusion Criteria

1. Admission to hospital with COVID-19 pneumonia
2. Pregnant, lactating or planning pregnancy during the course of the study
3. Known significant current or prior cardiorespiratory disease as determined at the post-COVID clinic, including significant smoking history (>10 pack years)
4. Known chronic renal impairment, with EGFR below 60 mL/min – this will be measured according to local hospital policy.
5. Known prior contrast media reaction
6. Inability to lie flat for imaging.
7. Contraindications to MRI examinations as locally determined.
8. COVID infection in the last 3 months
9. Any other reason, as determined by the study investigators, which renders the participant ineligible for the study.

Cohort C – Controls

Inclusion Criteria

1. Aged 18 years or over
2. Willing and able to give informed consent
3. Microbiological evidence of COVID-19 infection at any time since March 2020 – confirmed verbally by the participant
4. No on-going symptoms post COVID infection

Exclusion Criteria

1. Admission to hospital with COVID-19 pneumonia

2. Pregnant, lactating or planning pregnancy during the course of the study
3. Known significant current or prior cardiorespiratory disease including significant smoking history (>10 pack years)
4. Known chronic renal impairment, with EGFR below 60 mL/min – this will be measured according to local hospital policy.
5. Symptoms that fit with the definition of Long-COVID or 'Post-COVID syndrome' as defined by NICE 2020.
6. Known prior contrast media reaction
7. Inability to lie flat for imaging.
8. Contraindications to MRI examinations as locally determined.
9. COVID infection in the last 3 months
10. Any other reason, as determined by the study investigators, that renders the participant ineligible for the study.

9. PROTOCOL PROCEDURES

9.1. Recruitment

Sites involved in this study will be secondary care service providers in the NHS. Participants for Cohorts A & B will be recruited from Long-COVID clinics and for Cohort C via advertising.

9.2. Screening and Eligibility Assessment

For Cohort A and B, patients will be assessed by the clinical teams for eligibility against the inclusion or exclusion criteria. A member of the research team will contact the participant by telephone to discuss suitability for MRI scan. They may enquire if the potential participant has had a blood test in the last 3 months to check for kidney function – if they have not, they will be asked to have a blood test either at a GP or a hospital drive-through clinic.

The clinical teams at the recruiting sites will confirm that patients have been infected with COVID-19. Pre-screening for eligibility will take place prior to consent. It will be performed by the direct care team using patient notes. There will be no access to patient identifiable data outside of the direct care team prior to consent.

For cohort C, individuals responding to advertisements will be contacted by a member of the Research team, and will be screened to assess their suitability for inclusion by a telephone call which will run through the inclusion and exclusion criteria outlined earlier. They may be asked if they have had a blood test in the last 3 months to check for kidney function – if they have not, they will be asked to have a blood test either at a GP or a hospital drive-through clinic.

Participants must satisfy all the approved inclusion and exclusion criteria of the protocol. Participants will receive the Patient Information Sheet via email, or by post. They will then be invited to participate and informed consent will be obtained at the baseline visit, or at a visit prior to baseline scanning.

9.3. Informed Consent

The participant must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant-dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant.

Copies of the consent form will be retained at the study site, and will be stored with the participant hospital record for Cohort A and B.

9.4. Randomisation

This study is not randomised. At registration, all participants will be assigned a unique study ID.

9.5. Blinding and code-breaking

There is no blinding in the study, and no code breaking procedure.

9.6. Description of study intervention(s), comparators and study procedures (clinical)

This is a prospective, observational, cohort study of patients following COVID-19 infection.

9.6.1 Description of study intervention(s)

There will be no study intervention. This is an observational study.

9.6.2 Description of comparator(s)

There is no comparator in this study.

9.6.3 Description of study procedure(s):

Demographics, past medical and smoking (10 mins)

Medical history, demographics, smoking, allergies, medications and anthropometric measurements including height and weight, will be recorded during the study visit.

Observations (10-15 mins)

Blood pressure, heart rate and oxygen saturations

Renal Function Test (5 mins)

If local hospital policy calls for it, some participants will be required to have a blood test to check kidney function within 3 months of their scan.

Lung Function Test (30 Mins)

This will only be performed if not already done so in the 6 weeks prior to visit 1. Pulmonary lung function tests: Spirometry (FEV1 and FVC) and gas transfer (TLCO/KCO).

Exercise Test (10 mins)

A six-minute walk test will be performed where possible, otherwise a one-minute sit-to-stand test combined with pre and post oxygen saturations and mBORG score will be performed.

Questionnaires (45 mins)

Participant to complete questionnaires addressing dyspnoea, fatigue, quality of life and anxiety (Dyspnoea-12, Nijmegen Questionnaire, FACIT-F, GAD-7 and PHQ-9, Visual analogue fatigue rating, work and social adjustment scale and EQ-5D-5L.)

Low dose CT scan (15 mins)

All participants will have a low dose CT scan as part of the trial protocol, if this has not already been performed as part of their routine clinical care within the previous 6 weeks. Patients recruited for cohort A may have had a low dose CT scan as per current recommendations for unexplained post-COVID breathlessness, and this must be normal or nearly normal for them to be recruited (patients with post-COVID-19 ILD are being recruited into UKILD-PCF – Group 2, Cohort A). Cohorts B and C will have low dose CT scans as research scans. Only Group 2 from Cohort A will have a repeat CT scan, at 9 – 15 months post COVID-19 infection. Low dose CT has been shown to reduce radiation exposure, and has been approved previously by the HRA in healthy trial volunteers: the dose will be kept at or below previously HRA-approved exposure.

HPX-pMRI or HPX-MRI (approximately 30 mins)

We will perform hyperpolarised xenon MRI imaging on all enrolled participants, who will be asked to inhale hyperpolarised xenon for one breath hold, on up to 4 occasions per visit (this will enable different data to be acquired, and to allow scanning to be repeated if there is a technical failure). We will include a perfusion MRI (pMRI) using gadolinium contrast to detect areas of alveolar capillary thrombosis. Perfusion MRI has been shown to provide a safe non-ionising means of assessment of capillary perfusion and to be of equal sensitivity to SPECT perfusion imaging. Undertaken alongside dissolved phase xenon imaging, HPX, it will provide a means of assessment of the perfusion deficit in the capillaries and help establish whether breathlessness results from VQ mismatch, interstitial diffusion limitation or a combination of both. This will mean that we will be able to provide a subjective and quantitative method of assessing ventilation, diffusion and perfusion in NHLC patients.

Where a patient is contra-indicated to gadolinium contrast, or has opted out in the consent form, we will perform an HPX-MRI without perfusion. Any participant who is found to have abnormality on HPX-pMRI Chest will be followed up 3-6 months after baseline to repeat all the above assessments. If the HPX-pMRI Chest is normal, the participant will not have follow on imaging or any other tests. MRI scan protocols will be performed as per national agreed protocols for HPX-pMRI Chest in COVID-19 to enable combined data analysis and comparison across different studies.

<u>Hyperpolarised 129Xe</u>

Xenon is an inert gas that is present in the atmosphere in low concentrations. It has no smell or taste. Breathing xenon or undergoing an MRI scan does not involve exposure to ionising radiation. The xenon gas is manufactured and released in MHRA regulatory approved facilities at both Sheffield and Oxford.

Side effects

Xenon has been found to be extremely safe and well tolerated. Common side effects include: dizziness, drowsiness, deepening of the voice and a feeling of euphoria. They typically last less than a minute. In studies in over 1000 patients performed at both Oxford and Sheffield no SAEs have been reported related to xenon inhalation.

Gadolinium contrast

Contrast dyes (Gadolinium) are commonly used in MRI scans for medical care. They may occasionally cause mild rash, headache, and very rarely (less than 1 in 5000) a more severe allergic reaction. Although the risk of a severe reaction is small, there will be trained medical personnel present at all times with access to emergency equipment should this be required. In people with healthy kidneys, this dye is removed from the bloodstream within a few hours. Kidney function will be tested according to hospital policy.

Cardiac MRI (15 mins)

We will also aim to perform cardiac MRI in patients recruited to cohort B for scanning in Oxford, to determine whether HPX-pMRI Chest abnormalities are associated with cardiac impairment, which may potentially contribute to breathlessness. Cardiac MRI sequences will be brief and restricted to assessment of biventricular function, strain and strain rate.

9.7. Baseline and follow up assessments

Initial patient approach

Eligibility assessment by clinical team

Study information provided to eligible patients

Visit 1 Baseline (Participants post COVID-19 infection)

1. Review of eligibility with participant
2. Obtain written informed consent (unless obtained prior to baseline visit)

All procedures will be performed as listed above and as detailed in appendix B .

Visit 2 and 3 (3-12 months post baseline visit*) - abnormal* HPX-pMRI *results at baseline or Visit 2*

Participants will be invited to attend for follow up where all assessments from visit 1 will be repeated.

Visit 2 and 3 (3-12 months post baseline visit*) - normal results at baseline or at Visit 2*

Participants will be invited for remote follow up and asked to complete the same set of questionnaires as at visit 1.

Face to face visits will be scheduled to take place on 1 day – they will take approximately half the day.

9.8. Early Discontinuation/Withdrawal of Participants

During the course of the study a participant may choose to withdraw early from the study treatment at any time. This may happen for several reasons, including but not limited to:

- The occurrence of what the participant perceives as an intolerable AE
- Inability to comply with study procedures
- Participant decision

According to the design of the study, participants have the following options if they withdraw from the study:

1) Participants can withdraw from the study but permit data obtained up until the point of withdrawal to be retained for use in the study analysis. No further data would be collected after withdrawal.
2) Participants can withdraw from study scans but continue with data collection.

In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including, but not limited to:

- Pregnancy
- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Clinical decision

Participants that choose to withdraw from the study will not be replaced. The type of withdrawal and reason for withdrawal will be recorded in the CRF. If the participant is withdrawn due to an adverse event, the Investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

9.9. Definition of End of Study

The end of study will be defined as when all data has been entered and all queries resolved.

10. SAFETY REPORTING

10.1. Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of congenital anomaly or birth defect

Other ‘important medical events’ may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant 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.

10.2. Reporting Procedures for Serious Adverse Events

A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related' (resulted from administration of any of the research procedures) and 'unexpected' in relation to those procedures. Reports of related and unexpected SAEs should be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the HRA report of serious adverse event form (see HRA website). However, CT and Xenon MRI are widely used and we have established data on safety, therefore SAEs or related AEs are not expected.

11. STATISTICS AND ANALYSIS

11.1. Description of the Statistical Methods

All baseline characteristics and measures will be summarised overall and by group, using appropriate summary statistics.

We will describe the prevalence, quantitative and qualitative extent of persistent lung and cardiac-damage, in patients with COVID-19 disease and compare this with our matched controls. We will examine the relationship between extent of lung and cardiac injury, functional impairment and adverse clinical outcomes. We will compare quality of life and mental health scores in post SARS-CoV-2 infected patients and matched controls and assess its relationship with extent of lung and cardiac damage and initial hospital admission.

Number and proportions with HPX-pMRI Chest abnormalities will be summarised by group in order to estimate the sensitivity and specificity of the procedure. We will estimate the 95% confidence intervals for sensitivity and specificity. We will use logistic regression to build a predictive model of the relationship between presence of abnormalities and group with the aim of determining whether HPX-pMRI Chest abnormalities can discriminate between patients and controls. Mean Xenon detected gas transfer (RBC:TP) will be summarised by group and analyse using a linear regression model to estimate the mean difference between groups.

Numbers and proportions of participants with cardiac abnormalities detected will be summarised by group and presence of HPX-pMRI Chest abnormalities. Binary and categorical outcomes will be analysed using logistic regression.

Quality of life, mental health scores and frailty measures will be summarised by group and time point using mean and standard deviation (or median and interquartile range if non normally distributed).

Continuous measures, such as questionnaires, will be analysed using linear mixed models with fixed effects for group and timepoint (baseline visit and any follow up visits), as well as a group x timepoint interaction in order to determine the timepoint specific effects. A participant specific random effect will be fitted to account for clustering of timepoints within participants.

All models will be adjusted for baseline characteristics thought to be prognostic.

11.2. Sample Size Determination

Our previous data in patients enrolled in C-MORE POST- a sub-study of the HRA approved C-MORE study - demonstrated significant abnormalities in 18/22 scanned hospitalised patients. While we would not expect as high a proportion of abnormality within an NHLC patient cohort, the POST scan results provide important data on which to plan the sample size.
A sample size of 100 for the Cohort A is proposed, as this should be sufficient to perform multivariate analysis on the limited feature sets, and will be sufficient to assess the utility of hyperpolarised xenon to detect alterations if present in gas exchange in post-hospitalised patients.

Our planned sample size in Cohort B is 200 patients with NHLC and symptoms of breathless, 50 NHLC patients with no symptoms of breathlessness, and in Cohort C, 50 controls. Assuming a lower sensitivity of 70% in practice, then calculating for a 95% confidence interval and a width of 0.131 (equivalent to a lower limit of 63.1%) would require a sample size of 200 patients. If specificity is assumed to be 90%, then a sample size of 50 controls would give a confidence interval of width 0.185 (corresponding to a lower limit of 78.2%).

The mean value of Xenon detected gas transfer (RBC:TP) in patients was 0.3 (0.1) versus 0.6 (0.3) in healthy controls (absolute difference 0.3). This provides 90% power to detect a difference of 0.094 (based on a mean of 0.3 and SD of 0.1 in the control group and a SD of 0.3 in the Long-Covid group) in this key MRI parameter, which is well below the difference detected in POST. The level of statistical significance is set at 1.7% to allow for multiple comparisons between both patients and controls and patients with and without breathlessness.

11.3. Analysis populations

All participants who have completed HPX-pMRI scans will be included in the analyses.

11.4. Dissemination of Findings

Given the priority for dissemination of findings to inform public health and government bodies, interim descriptive results will be posted online after the first 50 participants then every 100 participants thereafter at the earliest time point of assessment.

11.5. Stopping rules

Not applicable.

11.6. The Level of Statistical Significance

All tests are two-tailed and p-values <0.05 were considered significant.

11.7. Procedure for Accounting for Missing, Unused, and Spurious Data.

The analysis models are valid under a missing at random (MAR) assumption. Baseline demographic variables will be summarised by availability of outcomes and any variables that appear to be predictive of missingness will be included in the analysis models in a sensitivity analysis. We do not expect any data to be missing at baseline but in the case of substantial (>10%) missing data at baseline multiple imputation including any variables predictive of missingness will be carried out as a sensitivity analysis.

For continuous measures outliers will be defined as any value that lies more than two standard deviations from the mean. We will explore the effect of excluding any outliers from the analysis in a sensitivity analysis. If the estimates do not differ substantively then the model retaining the outliers will be reported.

11.8. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Any deviations from the statistical analysis plan will be detailed along with the reason for the deviation in the statistical analysis report.

11.9. Health Economics Analysis

Not applicable.

12. DATA MANAGEMENT

The CT scans will be entered onto the hospital image archiving system, PACS, so they can be clinically reviewed and available for patient care by clinical teams. All image data will be pseudonymised at point of acquisition and transferred to Oxford University Hospitals NHS FT where it will be held on a secure server in the Oxford Radiology Research Unit, designed to hold information securely and in accordance with legal requirements. A copy of all pseudonymised MR images from sites will be shared with the

University of Sheffield, and will be held on a secure XNAT server at the University of Sheffield,which is the main site for analysis, and will contain no personal data.

All data transfer and data use will be in concordance with data protection regulation, Trust information governance procedures, in addition to the ethical and governance standards in the University of Oxford and the University of Sheffield. Access to the pseudonymised data will only be available to the relevant study teams and clinicians involved in the project.

The de-identified data from Oxford University Hospitals NHS FT may also be made available to third parties, as companies are working with the investigators to improve HPX-pMRI Chest imaging for patients with Long COVID and other lung diseases, subject to appropriate data sharing agreements.

All other pseudonymised data will be captured electronically using an electronic case report form (eCRF), Castor EDC, that meets data security and regulatory requirements (compliant to ISO27001). According to Castor EDC, all data is stored on servers hosted by certified hosting parties. In the UK, the servers are hosted by Microsoft Azure. Backups are made twice per day and moved to a separate geographic location daily. ORRU holds access to the study profile and can grant access for data entry only to the appropriate sites.

12.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely and securely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name, with the exception of the CRF, where participant initials may be added.

12.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

12.3. Data Recording and Record Keeping

Study data will be recorded in a pseudonymised manner and processed electronically where applicable; participants would not be identifiable from this. All participants will be identified by a code number (Study ID number) on electronic case report forms and any electronic databases i.e. their identity will remain unknown. Electronic cloud based software such as Castor EDC will be used to collect the study data.

This is an electronic data capture and management system that permits secure multi-site access. The server is based in the UK and complies with relevant laws to ensure that the data is held securely.

All source documents and questionnaires will have all identifiers removed and replaced by only the study ID number.

Those documents retaining personal identifiable data (e.g informed consent forms) will be stored securely in lockable cabinets and are only accessible by study staff and authorised personnel.

Personal data such as contact details and information that could identify a participant, (except for those who have consented to be approached for future research) will be destroyed as soon as it is practical to do so and no later than 12 months after the end of the study. During the study, the hard copy study data will be stored securely and at the end of the study, this will be archived in a secure commercial archive location with restricted access.

12.4 Data Sharing

Where the clinical team has verbal agreement from patients to share their contact details with the research team these contact details will be shared securely for example through NHS email accounts.

Pseudonymised imaging data will be shared from research sites to the sponsor via the Oxford University Hospitals NHS Foundation Trust secure XNAT server. This data will also be shared with Sheffield University for the purposes of analysis. There is a data transfer agreement in place detailing the relevant flows.

13. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

Data collected for the study may be reviewed for auditing and monitoring by authorised persons from the sponsor, regulatory authorities or their local host institutions to make sure that study is being carried out correctly. All investigators have a duty of confidentiality to research participants and nothing that could reveal their identity would be disclosed outside the research study team or site without specific consent from the research participant.

13.1. Risk assessment

A risk assessment and monitoring is not deemed necessary. Any risks associated with the study assessments or data management have been outlined in the protocol (sections 13 and 17.3) and mitigations outlined.

13.2. Study Monitoring

No monitoring required for this study.

13.3. Study Committees

13.3.1 Trial Management Group

Trial Management Group will meet regularly throughout the trial to discuss the day-to-day management of the trial. A TMG charter will be written detailing all the requirements.

13.3.2 Trial Steering Committee

A TSC will be convened to keep oversight of the trial. A charter will be written explaining the role of the TSC and each of its members. All members are required to sign a declaration of their participation. The charter will define how often the committee will meet during the course of the study.

13.3.3 ORTU Safety Oversight Group (SOG)

The Oxford Respiratory Trials Unit (ORTU) will conduct a review of all SAEs reported during the reporting period and cumulatively. The aims of this committee include:

- To pick up any trends, such as increases in un/expected events, and take appropriate action
- To seek additional advice or information from investigators where required
- To evaluate the risk of the trial continuing and take appropriate action where necessary

14. PROTOCOL DEVIATIONS

A study related deviation is a departure from the ethically approved study protocol or other study document or process (e.g. consent process or administration of study intervention) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the study master file.

15. SERIOUS BREACHES

A “serious breach” is a breach of the protocol or of the conditions or principles of Good Clinical Practice which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the trial subjects; or

(b) the scientific value of the research.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki. Guidelines for Good Clinical Practice. The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

16.2. Approvals

Following Sponsor approval, the protocol, the informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and HRA (where required) and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.3. Other Ethical Considerations

If any significant incidental unexpected abnormality is identified from blood tests or scanning, the participants primary or secondary care doctor will be informed dependent upon their route of enrolment.

16.4. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required), host organisation, Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties.

16.5. Transparency in Research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database such as ISRCTN.

16.6. Participant Confidentiality

The study will comply with the UK General Data Protection Regulation (UK GDPR) and Data Protection Act 2018, which require data to be anonymised as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

16.7. Expenses and Benefits

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate. There will be no direct benefit to participants,

although there maybe potential benefit if previously unknown health related findings were identified as part of the research assessments.

17. FINANCE AND INSURANCE

17.1. Funding

This study is funded by NIHR, the University of Oxford, the National Consortium of Intelligent Medical Imaging. .

17.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

17.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties. Site agreements will be put in place with NHS sites.

18. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the NIHR, NCIMI and other funding bodies that will continue to fund this study. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The University will ensure appropriate arrangements are in place as regards any new IP arising from the trial.

20. ARCHIVING

At the end of the study research files will be archived for 5 years and in line with local SOPs.

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46. Min L et al.

22. APPENDIX A: SUPPORTING DATA

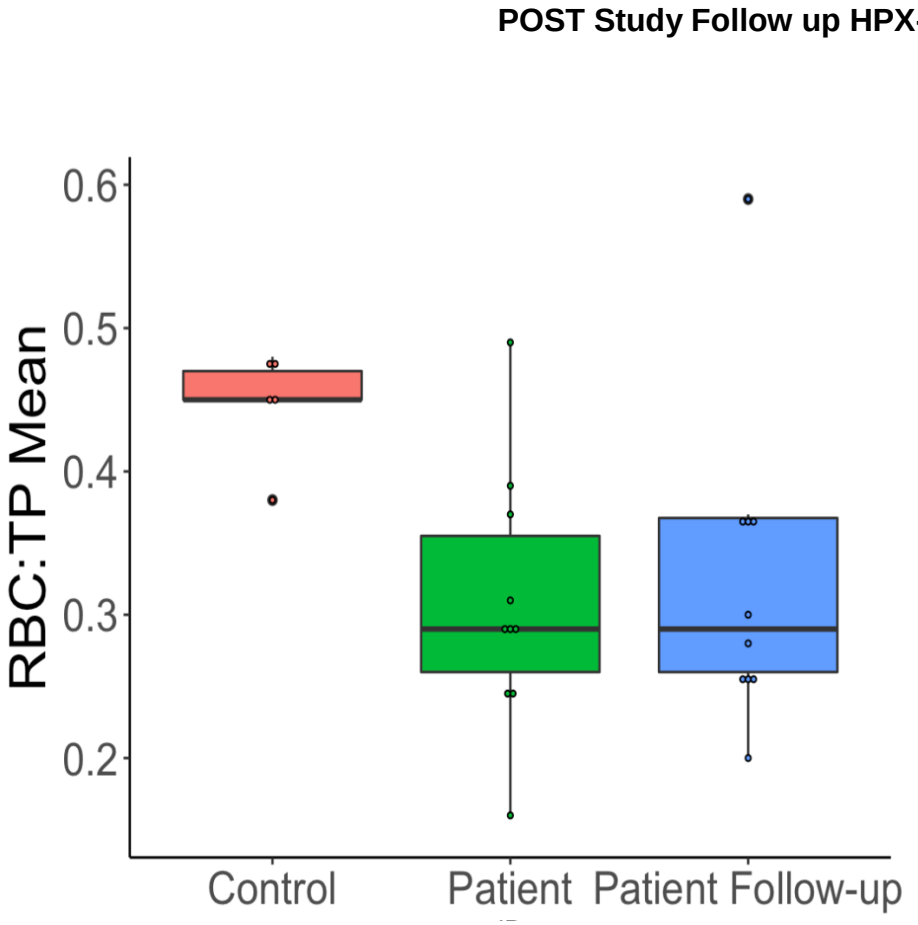

Figure 1: C-MORE POST[45] Sub-Study Follow up HPX-MRI data.

HPX-MRI RBC/TP data from the POST study

Showing:

- 5 controls with normal RBC/TP – lung diffusion
- 9 patients from POST
  - At baseline 3 months post discharge
  - At 6 month follow up

This shows that the majority 6/9 of the patients are not improving

The outlier has clearly improved, and was the least abnormal on baseline scanning

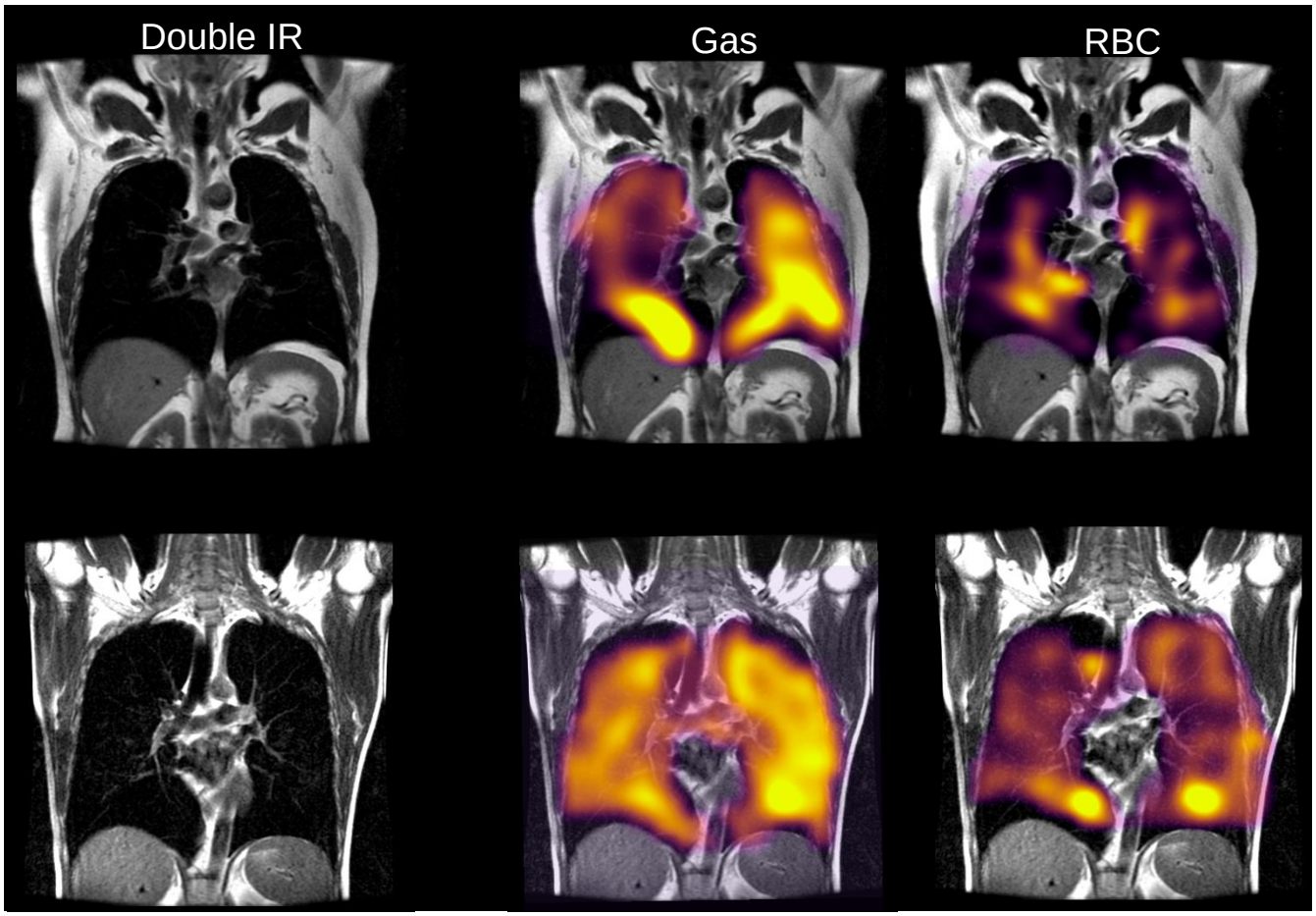

Figure 2: HPX-MRI scans in 2 NHLC patients.

The top row is a patient with ongoing breathlessness and the bottom row in a patient with improved breathlessness

23. APPENDIX B: SCHEDULE OF STUDY PROCEDURES

Cohort A	Hospitalised	Hospitalised	Hospitalised	Hospitalised
	All participants invited	Participants invited only if abnormal results at baseline	Participants invited only if normal results at baseline	Participants invited only if abnormal results at V2
Visit	**Visit 1 (Baseline)**	**Visit 2 (Follow-Up)**	**Visit 2 (Follow-Up) remote**	**Visit 3 (Follow-Up)**
Period	Day 0	6 months (-/+ 3 months)	3- 12 months	12 months (-/+ 3 months)
Eligibility assessment	✔			
Informed consent	✔ †			
Demographics, medical history, concomitant medications	✔			
Cardiorespiratory examination (incl. observations, heart rate, blood pressure and oxygen saturations)	✔	✔		✔
Blood test (Renal function)	✔ ††	✔ ††		✔ ††
Questionnaires	✔	✔	✔	✔
Lung Function Tests	✔*	✔*		✔*
Excercise test	✔	✔		✔
Low Dose CT	✔*	✔**		
MRI Chest	✔	✔		✔
MRI Chest with 129-Xenon gas	✔	✔		✔
MRI Chest with gadolinium contrast injection	✔	✔		✔

† Where appropriate, informed consent may take place prior to the baseline visit
†† In some locations, participants will have renal function testing performed according to local hospital policy.
*LFT and low dose CT to be performed only if they have not been performed clinically within 6 weeks prior to scan**Follow-up CT scan performed on Group 2 only.

Cohort B	Non-hospitalised	Non-hospitalised	Non-hospitalised	Non-hospitalised
	All participants invited	Participants invited only if abnormal results at baseline	Participants invited only if normal results at baseline	Participants invited only if abnormal results at V2
Visit	**Visit 1 (Baseline)**	**Visit 2 (Follow-Up)**	**Visit 2 (Follow-Up) remote**	**Visit 2 (Follow-Up) remote**
Period	Day 0	6 months (-/+ 3 months)	3- 12 months	12 months (-/+ 3 months)
Eligibility assessment	✔			
Informed consent	✔ †			
Demographics, medical history, concomitant medications	✔			
Cardiorespiratory examination (incl. observations, heart rate, blood pressure and oxygen saturations)	✔	✔		✔
Blood test (Renal function)	✔**	✔**		✔**

Questionnaires	✔	✔	✔	✔
Lung Function Tests	✔*	✔*		✔*
Exercise test	✔	✔		✔
Low Dose CT	✔*			
MRI Chest	✔	✔		✔
MRI Cardiac††	✔	✔		✔
MRI Chest with 129-Xenon gas	✔	✔		✔
MRI Chest with gadolinium contrast injection	✔	✔		✔

† Where appropriate, informed consent may take place prior to the baseline visit
†† Cardiac MRI to be performed in Oxford only
*LFT and low dose CT to be performed only if they have not been performed clinically within 6 weeks prior to scan** In some locations, participants will have renal function testing performed according to local hospital policy.

Cohort C	Controls
Visit	**Visit 1**
Period	Day 0
Eligibility assessment	✔
Informed consent	✔*
Demographics, medical history, concomitant medications	✔
Cardiorespiratory examination (incl. observations, heart rate, blood pressure and oxygen saturations)	✔
Blood test (Renal Function)	✔†
Lung Function Tests	✔
Excercise test	✔
Low Dose CT	✔
MRI Chest	✔
MRI Cardiac	✔**
MRI Chest with 129-Xenon gas	✔
MRI Chest with gadolinium contrast injection	✔

* Where appropriate, informed consent may take place prior to the baseline visit
** Cardiac MRI to be performed in Oxford only

† In some locations, participants will have renal function testing performed according to local hospital policy.

24. APPENDIX C: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2.0	30Mar2022	Emma Hedley	7. Removal of advertising email from the protocol. 12.4 Additional information added to the Data Sharing Section to include that imaging data will be shared with Sheffield University for analysis.
2	3.0	07Apr2022	Emma Hedley	8.2 clarification of inclusion criteria (Significantly breathless upon exertion) for groups 3, 4 and 5 (No respiratory symptoms) and Cohort C – controls (No on-going symptoms post COVID infection) in-keeping with the flow chart on page 19. Addition of an exclusion criteria “COVID infection in the last 3 months” for all groups. 9.8 Participants can withdraw from study scans but continue with data collection. 10. Removal of the word “an unexpected” in the SAE definition results in death