**Profiling the determinants of recovery to establish novel rehabilitation guidelines to improve clinically relevant and patient-reported outcomes in the post-COVID-19 period across 3 individual countries.**

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| **Background** | The value of clinical cardiopulmonary exercise testing (CPET) within healthcare settings has been established in the last decad. CPET methods remain highly relevant in the COVID-19 endemic phase and should be used to assess those recovering from COVID-19 infection. This diagnostic tool could play an integral role in disease prognostication and evaluate the integrative response to incremental exercise. Date from such assessments can enable practitioners to characterise cardio-respiratory fitness and identify reasons for physical impairment or abnormal cardio-respiratory function. More than 50% of patients admitted to hospital will experience cardiorespiratory issues and significant morbidity during their recovery and will require significant rehabilitative support. In this context, measurements obtained from an assessment of cardio-respiratory responses to physiological stress could provide insight regarding the integrity of the pulmonary-vascular interface and characterisation of any impairment or abnormal cardio-respiratory function. Current approaches to rehabilitation are being developed on existing knowledge from Severe Acute Respiratory Syndrome (SARS) and Acute Respiratory Distress Syndrome (ARDS) related illness. These provide important insight but do not provide insight into the novel challenges provided by COVID-19.<br><br>We bring together three clinical and academic research centres from around the world that collaborate as part of the Healthy Living for Pandemic Event Protection international network to conduct a minimum of 150 2-day CPET tests in patients that hospitalised with COVID-19. Patients will be engaged in testing in the UK (led by Faghy from the Human Science Research Centre, Derby and Maden-Wilkinson from the Research and Innovation for post-COVID-19 Rehabilitation centre, Sheffield), USA (led by Arena and Ozemek at the University of Illinois at Chicago) and India (led by Veluswamy and Babu at the Ramaiah Medical College, Bangalore). All tests will be conducted following standardised procedures documented by the American College of Sports Medicine. |
| **Acronym** | **HL-PIVOT-LCR** |
| **Short title** | HL-PIVOT: Long COVID Rehabilitation |
| **Chief Investigators** | Dr Mark Faghy (UK)<br>Dr Cemal Ozemek (USA)<br>Dr Sundar Kumar Veluswamy (India) |

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| **Aim** | Evaluate the integrative response to incremental exercise and increase diagnostic information relating to post-COVID-19 morbidity.<br><br>Assess cardio-respiratory responses to physiological stress and provide insight regarding the integrity of the pulmonary-vascular interface and characterization of any impairment or abnormal cardio-respiratory function. |
| **Trial Configuration** | Multi-Centre Observational Study |
| **Setting** | University Research Centre and Medical Facilities |
| **Number of participants** | 150 (n=50 per centre). |
| **Eligibility criteria** | **Eligibility**<br>• Aged 18 to 65 years old<br>• Diagnosed with COVID-19<br>• Still suffering from symptoms and experiencing functional limitations beyond 3 months after initial COVID-19 infection (quantified as PCFS grade 1 or greater).<br>• Sufficient English language comprehension and cognitive ability to understand the study protocol, give informed consent and follow instructions.<br><br>**Exclusion**<br>• <18 years of age<br>• Admitted to or received treatment from Intensive Care Units<br>• Unconfirmed COVID-19 test diagnosis<br>• Pregnancy<br>• Unable to perform cycle exercise.<br>• Unable to understand verbal or written information in English<br>• Achieving a grade 0 on the PCFS.<br>• Unstable angina<br>• Uncontrolled hypertension, that is, resting systolic blood pressure (SBP) >180mmHg, or resting diastolic blood pressure (BP) (DBP) >110mmHg<br>• Orthostatic blood pressure drop of >20 mmHg with symptoms<br>• Significant aortic stenosis (aortic valve area 120 bpm)<br>• Acute pericarditis or myocarditis |

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| | • Decompensated HF<br>• Third degree (complete) atrioventricular (AV) block without pacemaker<br>• Recent embolism<br>• Acute thrombophlebitis<br>• Resting ST segment displacement (>2 mm)<br>• Uncontrolled diabetes mellitus<br>• Severe orthopaedic conditions that would prohibit exercise<br>• Other metabolic conditions, such as acute thyroiditis, hypokalaemia, hyperkalaemia or hypovolaemia (until adequately treated)<br>• Severe grade 3 rejection (cardiac transplantation recipients Appendix N) |
| **Description of interventions** | Consecutive day 2 day CPET and follow up for 7 days for subjective feelings/fatigue. |
| **Duration of study** | Study Duration:<br>- Total trial duration 12 months<br>○ Recruitment 6 months (November 2022 to April 2023)<br>○ Data Collection (November 2022-April 2023)<br>○ Analysis and reporting: 4 months (April 2023-July 2023)<br>- Participant Duration: 3 sessions (1 Familiarisation, 2 CPET on consecutive days) and 1 week follow up |
| **Randomisation and blinding** | None |

| **Study Investigators** | |
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| **Chief Investigator:**<br>Dr Mark Faghy<br>Senior Lecturer in Exercise Physiology<br>School of Human Sciences<br>University of Derby, UK | **Principle Investigator (Sheffield, UK)**<br>Dr Tom Maden-Wilkinson<br>Senior Research Fellow<br>Advanced Wellbeing Research Centre<br>Sheffield Hallam University, UK |
| **Principle Investigator (Chicago, USA)**<br>Dr Cemal Ozemek<br>Clinical Associate Professor<br>Department of Physical Therapy<br>University of Illinois at Chicago<br>Chicago, USA | **Principle Investigator (Bengaluru, India)**<br>Dr Sundar Kumar Veluswamy<br>Assistant Professor, Department of Physiotherapy<br>Ramaiah Medical College, Bengaluru, India |
| **Co-Investigator:**<br>Dr. Ross Arena<br>Professor<br>Department of Physical Therapy<br>University of Illinois at Chicago<br>Chicago, USA | **Co-Investigator:**<br>Dr Ruth Ashton<br>Lecturer in Exercise Physiology<br>School of Human Sciences<br>University of Derby, UK |
| **Co-Investigator:**<br>Prof. Rob Copeland<br>Director, Advanced Wellbeing Research Centre<br>Sheffield Hallam University | **Co-Investigator:**<br>Dr Thomas Bewick<br>Consultant Respiratory Physician<br>University Hospitals of Derby and Burton<br>Derby, UK |
| **Co-Investigator:**<br>Dr Deepika Patel<br>Assistant Professor<br>Department of Physical Therapy<br>University of Illinois at Chicago<br>Chicago, USA | **Co-Investigator:**<br>Dr Emma Sharpe<br>School of Human Sciences<br>University of Derby |
| **Co-Investigator:**<br>Mr Alex Bugg<br>Researcher, Advanced Wellbeing Research Centre, Sheffield Hallam University. | **Co-Investigator:**<br>Dr Simon Goodwill, Principal Research Fellow, Advanced Wellbeing Research Centre, Sheffield Hallam University. |
| **Co-Investigator:**<br>Dr Caroline Dalton, Associate Professor, Advanced Wellbeing Research Centre, Sheffield Hallam University. | **Co-Investigator:** |

# Contents

## 1.0 Background

The value of clinical cardiopulmonary exercise testing (CPET) within healthcare settings has been established in the last decade [5]. CPET methods remain highly relevant in the COVID-19 endemic phase and should be used to assess those recovering from COVID-19 infection. This diagnostic tool could play an integral role in disease prognostication and evaluate the integrative response to incremental exercise. Date from such assessments can enable practitioners to characterise cardio-respiratory fitness and identify reasons for physical impairment or abnormal cardio-respiratory function. More than 50% of patients admitted to hospital will experience cardiorespiratory issues and significant morbidity during their recovery and will require significant rehabilitative support. In this context, measurements obtained from an assessment of cardio-respiratory responses to physiological stress could provide insight regarding the integrity of the pulmonary-vascular interface and characterisation of any impairment or abnormal cardio-respiratory function. Current approaches to rehabilitation are being developed on existing knowledge from Severe Acute Respiratory Syndrome (SARS) and Acute Respiratory Distress Syndrome (ARDS) related illness. These provide important insight but do not provide insight into the novel challenges provided by COVID-19.

We bring together three clinical and academic research centres from around the world that collaborate as part of the Healthy Living for Pandemic Event Protection international network to conduct a minimum of 150 2-day CPET tests in patients that hospitalised with COVID-19. Patients will be engaged in testing in the UK (led by Faghy from the Human Science Research Centre, Derby and Maden-Wilkinson from the Research and Innovation for post-COVID-19 Rehabilitation centre, Sheffield), USA (led by Arena and Ozemek at the University of Illinois at Chicago) and India (led by Veluswamy and Babu at the Ramaiah Medical College, Bangalore). All tests will be conducted following standardised procedures documented by the American College of Sports Medicine.

## 2.0 Aims and Objectives

- Evaluate the integrative response to incremental exercise and increase diagnostic information relating to post-COVID-19 morbidity.
- Assess cardio-respiratory responses to physiological stress and provide insight regarding the integrity of the pulmonary-vascular interface and characterization of any impairment or abnormal cardio-respiratory function.

### 2.1 Primary Aim

- Evaluate the integrative response to incremental exercise and increase diagnostic information relating to post-COVID-19 morbidity.

### 2.2 Secondary Aims

- Examine the relationship between COVID-19 symptom prevalence and exercise tolerance
- Examine the prevalence of post-exertional malaise in people with long covid.
- Examine the role of vaccination history (COVID-19) on exercise tolerance.

- Assess cardio-respiratory responses to physiological stress and provide insight regarding the integrity of the pulmonary-vascular interface and characterization of any impairment or abnormal cardio-respiratory function.

# 3.0 Study Design

A multicentre, cross-sectional, observational study.

## 3.1 Study Sites

**UK Sites:**

The study and data collection will be conducted at the following sites:

- University of Derby Kedleston Road, Derby, DE22 1GB
- Sheffield Hallam University, Olympic Legacy Park, Sheffield, S9 3TY
- Northumbria University, Northumberland Building, Newcastle upon Tyne, NE1 8ST
- University of Exeter, Stocker Rd, Exeter, EX4 4PY

**International Sites:**

**Ramaiah Medical College, Bengaluru, India**
In India, the study will be conducted at the Centre for Rehabilitation, and the Department of Physiotherapy at the Ramaiah Medical College Hospitals. Patients will be recruited through telephonic follow-up of patients discharged from the Ramaiah Medical College Hospitals as well as through the Post COVID Clinic of at Ramaiah Memorial Hospital.

**University of Illnois, Chicago.**
In the USA, study visits will be conducted at the University of Illinois' Physical Therapy Faculty Practice, Chicago, IL, USA. Patients will be recruited from the University of Illinois Hospital through the pulmonology and cardiology clinics as well as through word of mouth and social media.

## 3.2 Sample Size

A minimum of 150 patients admitted to hospital with a positive COVID-19 infection will be recruited for WP2. Patients for WP2 will be recruited to the study from three international centres (UK; led by Faghy, Royal Derby and Burton Hospital Trust and the Research and Innovation for post-COVID-19 Rehabilitation Centre; USA led by Arena, University of Illinois at Chicago; India led by Veluswamy , Ramaiah Medical College, Bengaluru. In line with the consensus in current literature, patients will not be treated in intensive care units. Those requiring rigorous treatment via intensive care units will experience profound complications in the post-COVID-19 period and trials involving these groups have already been established by clinical centres (Imperial

College London). Currently the Office for National Statistics estimates that 1.2 million people in the UK are suffering with Long COVID.

## 3.3 Eligibility and Exclusion

Eligibility criteria includes:

- Aged 18 to 65 years old
- Diagnosed with COVID-19
- Still suffering from symptoms of COVID-19, 3 months after initial infection
- Sufficient English language comprehension and cognitive ability to understand the study protocol, give informed consent and follow instructions.

Exclusion criteria includes:

- <18 years of age
- Admitted to or received treatment from Intensive Care Units
- Unconfirmed COVID-19 test diagnosis
- Unable to understand verbal or written information in English
- Achieving a grade 0 or 1 on the PCFS.
- Unstable angina
- Uncontrolled hypertension, that is, resting systolic blood pressure (SBP) >180mmHg, or resting diastolic blood pressure (BP) (DBP) >110mmHg
- Orthostatic blood pressure drop of >20 mmHg with symptoms
- Significant aortic stenosis (aortic valve area 120 bpm)
- Acute pericarditis or myocarditis
- Decompensated HF
- Third degree (complete) atrioventricular (AV) block without pacemaker
- Recent embolism
- Acute thrombophlebitis
- Resting ST segment displacement (>2 mm)
- Uncontrolled diabetes mellitus
- Severe orthopaedic conditions that would prohibit exercise
- Other metabolic conditions, such as acute thyroiditis, hypokalaemia, hyperkalaemia or hypovolaemia (until adequately treated)
- Severe grade 3 rejection (cardiac transplantation recipients Appendix N).

## 3.4 Approach/Recruitment

Participants will be recruited from existing patient databases and Long COVID clinics in the UK as well as through social media.

## 3.5 Ethical Considerations

### *3.5.1 Consent*

Eligible patients will be identified at the designated PIC sites (STH, Sheffield; UIC, USA and Ramaiah, India) by individuals knowledgeable about the research. The researcher will introduce themselves to the patient and/or patient representatives and explain the nature of their visit to the patient. The researcher will proceed, if the patient agrees, to discuss the nature, objective, risks, and benefits of the proposed research. This will include the provision of written information regarding the research study, implications, expected outcomes, risks, and benefits. The patient will be allowed to ask questions throughout this process.

Once patients have indicated their willingness to participate in the research, they will be invited to a session at their local site. Consent will be obtained by members of the research team and documented using the study consent form. Informed consent will be obtained before the participant undergoing any activities that are specifically for the study. All study documentation (i.e. participant information sheet, participant consent form) will be kept in the Trial Master File.

The right of the patient to refuse to participate in the study without giving any reason must be respected. Equally, the patient will be free to withdraw their consent to participate in the study without providing any reasons and without prejudicing his or her further treatment.

### *3.5.2 Deception*

No deception will occur within this project. All research aims and procedures will be articulated to the patient and included within the PIS.

### *3.5.3 Debriefing*

All participants will be given the opportunity to hear about the results of the study in the form a short summary upon completion. This will be provided by the chief investigator and all participants will be able to 'opt out' of this opportunity should they wish to.

Regardless all participants will be thanked for their participation at the final visit.

### *3.5.4 Withdrawal from the investigation*

Patients will be free to withdraw their consent to participate in the study at any time without providing any reasons and without prejudicing his or her further treatment.

Withdrawal from the investigation can be done at any time by contacting the chief investigator and/or members of the study team using the contact details provided on the PIS.

*3.5.5 Anonymity and Confidentiality*

Information related to participating in the study will be kept confidential and managed in accordance with the Data Protection Act, NHS Caldecott Principles, The Research Governance Framework for Health and Social Care and the conditions of Research Ethics Committee approval.

Staff involved in the study will ensure that all participants' confidentiality is maintained at all times.

On all study documentation participants will be identified only by a unique patient study identification number on the CRF and central electronic database (Redcap).

*3.5.6 Protection of Participants*

All components of the research activity has been approved by local NHS REC and the HRA (via the IRAS system) and all planned activities have also been approved by the clinical research support team. All participants will be informed and aware of the expectations taking part in the research and will be provided with regular opportunity to ask questions relating to their involvement.

*3.5.7 Observation Research*

This study will comprise of a 2-week cohort observation. Whilst the study design is adopting an observational approach participants will be made aware of all research activities.

*3.5.8 Giving Advice*

No advice will be provided by the research team. If patients raise questions relating to clinical issues relating to their health, they will be directed to
contact their GP.

The PIS also provides links to NHS helpline/advice pages which patients will be directed too.

Research will not be undertaken in public places.

*3.5.9 GDPR - Collecting Personal Data*

Researchers will be collecting data from participants in this study. This data has been specifically chosen to achieve the aforementioned aims and study outcomes and in the public interest of enhancing clinical understanding academic research. No information that is not directly associated with the research questions will be collected.

This is the legal basis on which we are collecting your data and while this allows us to use your data, it also means we have obligations towards you to:

- Not seek more information from you than what is essential and necessary for the study;
- Make sure that you are not identified by the data by anonymising it using ID codes;
- Use your anonymised data only for the purposes of this study and for any relevant publications that arise from it;

- Store data safely in password-protected databases to which only the named researchers have access;
- Not keep your information for longer than is necessary (usually for seven years);
- Safely destroy your data by shredding or permanently deleting them.
- Researchers on the project with access to the data are supervised by highly qualified and experienced staff and have been very careful to ensure the security of your data.

The study was approved for its ethical standards by Health Research Authority (IRAS 313936) and the University of Derby Human Sciences Research Ethics Committee.

*3.5.10 Basis for Collecting Data*
Consent

*3.5.11 Data Retention*
Study records will be stored for 15 years according to the Research & Innovation Office SOP for archiving, in a secure off-site storage. Direct access to source documents and data will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits, inspections and reviews.

*3.5.12 Rights of Data Subject*
All rights have been addressed via the utilisation of the GCP guidelines and via HRA approval.

# 4.0 Study Protocol

Each test will be interpreted following published guidelines provided by the American College of Sports Medicine. Upon the conclusion of each test, the key descriptive characteristics and findings will be recorded to facilitate the use of cohort analyses. Data will be stratified according to the demographic (age, gender, ethnicity, pre-COVID-19 functional status and important physiological parameters for a given workload (absolute and relative oxygen consumption or $VO_2$ peak, $O_2$ pulse, alveolar-arterial O2 gradient, arterial to end-tidal CO2 difference and the relationship between carbon dioxide output and ventilation ($V_E/VCO_2$ slope)). These approaches will be used to identify differences, trends and patterns in key defining variables that might lead to longstanding impairment/morbidity. Study day 1 will be conducted 7-10 days post baseline measures to ensure no symptoms of post exertional malaise and/or excessive fatigue from baseline assessments. Patients will wear accelerometers during this period to compare baseline and follow up.

| Baseline | Session 2 | Session 3 (+1 day) | 7 Day follow up |
|---|---|---|---|
| Anthropometrics | Symptom Profile* | Symptom Profile* | Accelerometery |
| Questionnaires | ECG | ECG | Patient Log/Diary |
| Symptom Profile* | Resting HR + BP | Resting HR + BP | HRV |
| Lung Function | CPET Lactate and BP | CPET Lactate and BP | |
| TUG | Recovery following | Recovery following | |
| 6MWT | CPET | CPET | |
| | HRV | HRV | |

## 4.1 Baseline Clinical Data

Data collected will include demographics, the pre-admission symptoms, pre-admission therapy, preadmission exercise tolerance and performance status (retrospective assessment by the patient and/or representative 6 weeks preceding admission) level of educational attainment, past-medical history, time and route of admission, and in-hospital routine physiological observations (including heart rate, blood pressure, oxygen saturation, respiratory rate and temperature). Blood biomarkers that are part of clinical profiling such as CRP, total blood count, D-Dimers and IL-6 as well as exploratory markers for Long covid mechanisms including inflammatory responses and clotting factors. This will also be performed pre and post (30mins) exercise bouts..

### *4.1.1 Anthropometrics (Session 1)*

Participants will be asked to provide an approximate date of onset of COVID-19 and whether they have received any COVID-19 vaccinations and the manufacturer of the vaccine. Body Mass (kg) and height (m) will be assessed.

### *4.1.2 Questionnaires*

#### 4.1.2.1 Symptom Profile

Symptom profile will be measured twice at the baseline assessment (Day 0), to capture a) retrospective assessment by the patient of their symptom status at symptom onset (essentially their baseline symptoms), and b) their current symptom status at the point of testing.

#### 4.1.2.2 Quality of Life

The EQ-5D-5L is routinely used in the assessment of the quality of life in respiratory research and is available in more than 130 languages (EQ-5D-5L). It comprises five dimensions (Previously 3): mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has five levels: no problems, slight problems, moderate problems, severe problems, and extreme problems.

#### 4.1.2.3 Sleep Behaviour

Participants will complete the Pittsburgh Sleep Quality Index (PSQI). The PSQI is a self-report measure to assess sleep quality over a 4 week in period, throughout the

intervention. The measure consists of 19 individual items, with a 0-3 interval scale and creates 7 components that produce one global score. The PSQI is reliable and valid in the assessment of sleep problems.

#### 4.1.2.4 Fatigue

Fatigue is a common complaint in patients recovering from an acute respiratory infection and is not adequately captured in general quality of life or specific recovery questionnaires. Participants will complete 2 separate measures of fatigue at Visit 1 and Visit 2); (A) the Fatigue Assessment Scale (FAS), a self-reported questionnaire validated to assess presence and severity of fatigue; and (B) MFI-20. A 20 item self-reported questionnaire assessing fatigue across five aspects.

#### 4.1.2.5 MRC Dyspnoea Scale

The Medical Research Council (MRC) Dyspnoea Scale (MRC) is a measure to grade the effect of breathlessness on the person's daily activities. The MRC is simple to administer and is a short 1-5 stage scale (1 item) and allows the person to state the extent to which their breathlessness affects their mobility.

#### 4.1.2.6 Post-COVID-19 Functional Status (PCFS) Scale

The Post-COVID-19 Functional Status (PCFS) Scale will be assessed upon discharge from the hospital and during each face-to-face visit to monitor direct recovery and to assess functional sequelae. The PCFS will evaluate the ultimate consequences of COVID-19 on functional status and supplement other instruments that measure quality of life, tiredness, or dyspnoea in the acute phase. The PCFS covers the full spectrum of functional outcomes and focuses on both limitations in usual duties/activities and changes in lifestyle in six scale grades. Briefly grade 0 reflects the absence of any functional limitation, and the death of a patient is recorded in grade D. Upward of grade 1, symptoms, pain or anxiety are present to an increasing degree. This has no effect on activities for patients in grade 1, whereas a lower intensity of the activities is required for those in grade 2. Grade 3 accounts for inability to perform certain activities forcing patients to structurally modify these. Finally, grade 4 is reserved for those patients with severe functional limitations requiring assistance with activities of daily living (ADL).

#### 4.1.2.7 Cognition

The Montreal Cognitive Assessment (MoCA) will be conducted at Visit 1. To allow accurate interpretation, highest educational level/attainment will be recorded during data collection

### *4.1.3 Functional Status*

Two different assessments of lower extremity functional capacity will be conducted at each visit these are (A) the 6-minute walk test (6MWT) and (B) the timed up and go test (TUG). The 6MWT is a standardised and widely used measure of functional status in individuals with chronic disease such as chronic obstructive pulmonary disease, cystic fibrosis, congestive cardiac failure, peripheral vascular disease, and elderly. It has been previously used to assess response to interventions and predict morbidity and mortality. Participants will be given a shortened practice attempt (1-2 minutes) at the beginning of each of their assessment visits. This will not be recorded but will be performed to allow familiarisation with the assessment and repeated at each visit for

consistency. Patients suffering from excessive fatigue/Post exertional malaise from the 6MWT will be excluded from the study to prevent further onset of these symptoms.

The TUG is reliable and reproducible and has been validated as a predictor of frailty and risk of falls in elderly adults. The TUG has several advantages over other measures, mainly its reproducibility and shorter assessment time. Its use at predicting functional status following pneumonia has not been assessed previously.

Participants will also be asked to self-report their return to functional activity and employment at each contact with the research team (telephone consultations and follow-up visits.) Each participant will be asked at each contact with the research team if they have returned to perform their activities of daily living to the same level as 6 weeks before admission, and if they have returned to their occupation (if employed.) The patient-reported date of return to these activities will also be recorded.

### *4.1.4 Lung Function (Session 1)*

Full lung function tests including FEV1, FVC, PEFR, flow-volume curves, MIP, and MEP will be conducted during first visit. These spirometry measurements will be conducted by a suitably qualified individual, proficient in the use of the specific equipment and according to published standards.

#### *4.1.5 Electrocardiogram (ECG) and Blood Pressure (Session 2 and 3)*

Participants will then be fitted with: a 12-channel electrocardiography (ECG) device (Custo Cardio 200, Customed, Ottobrunn, Germany); a blood pressure cuff positioned over the brachial artery (Tango M2, SunTech, Eynsham, UK). Measurements will be taken following 10 minutes of supine rest, during sit to stand task and monitored continuously throughout the CPET.

#### *4.1.6 Cardiopulmonary Exercise Test (CPET) (Session 2 and 3)*

Cardiopulmonary exercise testing will be performed to measure VO2 peak and other parameters representative of cardiovascular reserve (McGregor et al., 2016). Tests will be conducted using a standard bicycle ramp protocol in accordance with American Thoracic Society guideline (Ross et al., 2003). In accordance with pilot data, we will stratify individuals starting load based on their 6MWT distance, Strata I: 6MWD < 350 (starting load of 10 watts; with subsequent increments of 5 watts), Strata II: 6MWD 350 – 400 (starting load of 20w with subsequent increments of 5w and Strata III: 6MWD > 400 (starting load of 30w with subsequent increments of 5W)

Participants will then complete a maximal graded exercise test on a friction-loaded cycle ergometer (Monark 894E Ergomedic Peak Bike, Monark, Varberg, Sweden). The cycle ergometer friction wheel will be calibrated according to manufacturer recommendations prior to the study. The cycle ergometer will be set up according to each participant's body size and personal preference. The knee angle at the bottom of the pedal stroke will be ~25°, which is optimal for movement economy and injury prevention. Knee angle will be measured using a universal goniometer. The cycle ergometer handlebars will be positioned so that the participant can comfortably maintain an upright posture. The cycle ergometer set-up will be identical for each visit.

A step-wise incremental exercise test will be performed at a cadence of 60 revolutions per minute (rpm) (±10%). The exercise protocol will begin with a 3-minute rest period, followed by a step-wise incremental protocol, designed to achieve volitional exhaustion within 8-12 minutes of exercise onset. The exercise protocol will be individualised based on participants' predicted exercise capacity, as described above. Heart rate and pulmonary gas exchange data will be measured continuously. Blood pressure and rating of perceived exertion (RPE) (6 to 20 scale) will be measured during the final 15 second period of each minute. For blood pressure measurements, participants will be asked to relax their arm and shoulder muscles whilst their forearm is rested on the researcher's shoulder (padded with a folded towel). This will ensure that the participant's arm is positioned near heart level to reduce measurement error. At the end of the exercise test, participants will complete a cooldown consisting of unloaded pedalling at 20rpm for 5-10 minutes. All data will be exported for offline analysis using Microsoft Excel (Washington, USA).

Criteria for the assessment of a good participant effort will include peak respiratory exchange ratio (RER) >1.10, peak HR≥85% predicted and RPE ≥18.31 (Balady et al., 2010). Prior to the test, participants will be monitored for 5 minutes to allow passive data collection and for a total of 10 minutes after completion.
We will analyse over 30s averages to allow for differences in Gas analysis systems and monitor heart rate throughout test and 10 minutes seated recovery. Blood Pressure monitoring continuously (every 30s). Blood lactate concentration will be monitored through fingertip sampling at the end of each stage and at 5-minute intervals for 30 minutes following volitional fatigue.

#### 4.1.6.1 Pulmonary Gas Exchange Measures

Breath-by-breath pulmonary gas exchange data will be smoothed using a middle five of seven breath average. Key variables of interest include: the first ventilatory threshold ($VT_1$), the respiratory compensation point (RCP), peak oxygen consumption ($\dot{V}O_{2peak}$) and end-tidal $CO_2$. $\dot{V}O_{2peak}$ is defined as the mean $\dot{V}O_2$ over the last 30 seconds of the exercise test, adjusted for body mass ($ml \cdot kg^{-1} \cdot min^{-1}$). $VT_1$ will be determined by using the V-slope method to identify the deflection point in the relationship between $\dot{V}CO_2$ and $\dot{V}O_2$ – indicating a disproportionate rise in $CO_2$ production due to increasing anaerobic glycolytic activity (65). $VT_1$ will be confirmed using the nadir of the ventilatory equivalent (VE) for $\dot{V}O_2$ ($VE/\dot{V}O_2$) (65). The RCP will be determined using the V-slope method to identify the deflection point in the relationship between VE and $\dot{V}CO_2$ – which marks the onset of hyperventilation caused by excessive blood lactate accumulation (65). The RCP will be confirmed using the nadir of $VE/\dot{V}CO_2$ (65). The $VT_1$ and the RCP will be confirmed by two researchers at each site. Where agreement is not reached, a third researcher will adjudicate. End-tidal $CO_2$ is the partial pressure of $CO_2$ measured at the end of each breath.

| **Exercise termination criteria** |
|---|
| Chest pain suggestive of ischaemia |
| Fall in systolic blood pressure >20 mmHg from the highest value during exercise |
| >225 mmHg systolic blood pressure |
| >130 mmHg diastolic blood pressure |
| Hypotension (<100 mmHg systolic) |

| |
|---|
| Severe desaturation: $SpO_2 \leqslant 80\%$ when accompanied by symptoms of severe hypoxaemia |
| Sudden pallor |
| Loss of coordination |
| Mental confusion |
| Dizziness or faintness |
| Signs of respiratory failure |

## 4.2 Follow-up Assessments

Using a custom developed application alongside commercially available heart rate monitoring devices, participants will be asked to provide accelerometery (asked through the participants phone), heart rate variability (iHeart) and a subjective wellness score for each day following baseline and CPET investigations each for a period of 3 days. This custom developed software has been developed at the Advanced Wellbeing Research Centre to provide objective and subjective assessment of fatigue in people suffering with long covid and has successfully been piloted with over 200 individuals. Where smartphone data is not possible, individuals will be asked to wear an accelerometer and subjectively report their wellness through patient diary.

## 4.3 Safety of Graded Exercise Testing

Graded exercise testing is a safe procedure which is routinely used in clinical, exercise and research settings. Numerous studies that have assessed the safety of graded exercise testing (mostly in cardiac patients) indicate that the risk of exercise-induced complications and mortality is very low. The reported mortality rate ranges from 0 to 1 death per 10,000 tests, and the non-fatal adverse event rate ranges from 0 to 5.2 per 10,000 tests.

## 4.4 Risk Management

### *4.4.1 Cardiovascular Risk*

Participation in aerobic exercise may increase the risk of cardiovascular complications such as cardiac ischaemia, arrythmia or hypotension. The use of an appropriate graduated warm-up and cool-down is recommended to minimise these risks (ACSM, 2021). In the proposed study, participants' physiological response to exercise will be closely monitored during and after exercise. Exercise will be stopped if any adverse signs or symptoms occur (see table above). The researcher is experienced in the provision of clinical exercise tests and is trained in cardiopulmonary resuscitation.

### *4.4.2 Musculoskeletal Risk*

Participation in aerobic exercise may increase the risk of musculoskeletal injury such as a muscle strain. This risk will be minimised by conducting a graduated warm-up and by following the cycle ergometer manufacturer guidelines regarding correct positioning of participants.

### *4.4.3 Post-Exertional Malaise*

Post exertional malaise has been identified in patients with long covid especially with incremental exercise and exertion. We will screen for individuals' experiences PEM

prior to the study and follow up with individual participants following baseline measurement sessions before proceeding with repeated day CPET. Those individuals who show symptoms of PEM will be provided with guidance of pacing and provided with access to published materials around managing fatigue as per UK NHS long covid clinic guidance (World Physiotherapy, 2021).

#### 4.4.4. Bruising.

Bruising may occur following venepuncture sampling, participants will be advised of this through PIS and management if bruising occurs (Rest and cold exposure).

### 4.5 Statistical Analysis

Statistical analysis will be supervised by Dr Emma Sharpe at the University of Derby and conducted using SPSS version 26. This study is a prospective observational cohort study, designed to identify suitable tools and measures across different domains for assessing patient recovery from COVID-19.

Descriptive statistics will be calculated for all outcomes of interest. These will be presented as proportions and means with standard deviations or medians with interquartile ranges, depending on the distribution of data.

Within group differences will be compared using parametric (students t-test) and non-parametric (Mann-Whitney U, Chi square) tests, depending on normality of distribution.

### 4.6 Sponsor and Coordination

The University of Derby, UK will act as the sponsor for the research and Dr Mark Faghy will act as the Chief Investigator. With site principal investigators as follows:

- Advanced Wellbeing Research Centre, Sheffield UK - Dr Tom Maden-Wilkinson
- Ramaiah Medical College, Bengaluru, USA - Dr Sundar Kumar Veluswamy
- University of Illinois, Chicago - Dr Cemal Ozemek

### 4.7 Adverse Events and Reporting

#### *4.7.1 Definition of Adverse Event (AE)*

Any untoward medical occurrence in a clinical study where participants are administered a medicinal product, which does not necessarily have to have a causal relationship with participation in this study.

An AE includes:

- Exacerbation of pre-existing illness
- Increase in frequency or intensity of a pre-existing episodic event or condition
- Condition detected or diagnosed after study intervention even though it may have been present before the start of the study
- Continuous persistent disease or symptoms present at baseline that worsens following the start of the study

An AE does not include:

- Medical or surgical procedure (e.g. surgery, tooth extraction) but the condition that leads to the procedure is an AE
- Pre-existing disease or conditions present or detected at the start of the study that did not worsen
- Situations where an untoward medical occurrence has occurred (e.g. hospitalisations for cosmetic elective surgery)
- Disease or disorder being studied, or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition
- Overdose of concurrent medication without any signs or symptoms

*4.7.2 Non-serious AEs*

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

*4.7.3 Abnormal Baseline Findings*

Abnormal findings at that are deemed to be a clinically significant abnormality may be reported as an AE, according to the judgement of the Chief Investigator taking into account any associated clinical symptoms/signs from the below measures during pre; mid and post time frame.

*4.7.4 Reporting Procedures for All AEs*

Aim: to describe adverse events relating to study and the reporting process

Adverse event (AE) and Serious Adverse Event (SAE) will use the local NHS Trust reporting procedures but reported to the University of Derby Research and Knowledge Exchange Office as soon as possible.

All AEs should be reported on the CRF. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance. Participants will be asked to report AE's and SAE's during all follow up sessions. All participants will be provided with contact details for a trial phone, where they can communicate AE/SAE throughout the study and will be operated during working hours (Monday to Friday 9-5). Outside of these working hours, participants are encouraged to leave a voicemail and the study team will contact them back as soon as possible during working hours.

The following information will be recorded: description, date of onset and end date and severity. Follow-up information should be provided, as necessary. AEs considered related to the study as judged by a medically qualified investigator (Tom Bewick, CI) or the sponsor will be followed until resolution or the event is considered stable. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs. It will be to the responsibility of the Chief Investigator's clinical judgement whether an AE is of sufficient severity to warrant removal from the study. The relationship of AEs to the study will be assessed by a medically qualified investigator. All such events, whether expected or not will be recorded.

*4.7.5 Definition of 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

An SAE form will be completed and forwarded to the sponsor or delegated representative within 24 hours. However, relapse and death and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs. All SAEs should be reported to the study sponsor local Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

- '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

*4.7.6 Reporting Procedures for All SAEs*

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the 'COREC SAE' form for non-CTIMP studies. Local investigators should report any SAEs as required by their Local Research Ethics Committee and/or Research & Development Office. Fatal or life-threatening SAEs must be reported within 7 days which will be reported to the Competent Authorities MHRA and the Research Ethics Committee.

SAE reports must be sent to R&I using one of the following methods:

i. Email: UKREO@Derby.ac.uk
ii. Hand Delivered (Not Mailed): South Tower, Kedleston Road
iii. Telephone: 01332 59 7815 (If written report not immediately possible)

The intensity of the AE will initially be assessed according to the following definitions:

- *Mild*: An event easily tolerated by the participant, causing minimal discomfort, and not interfering with everyday activities
- *Moderate*: An event sufficiently discomforting to interfere with everyday activities
- *Severe:* An event that prevents everyday activities

All AE and SAE Reporting documentation (i.e. NHS National Patient Safety Agency, National Research Ethics Service, Report of Adverse Event (AE) and Serious Adverse Event) physical copies of these will be kept in the Trial Master File.

# 5.0 Data Protection and Patient Confidentiality

All investigators and study site staff will comply with the requirements of the Data Protection Act 2018 with regards to the collection, storage, processing, and disclosure of personal information and will uphold the Act's core principles. This includes a trial independent monitor which will carry out the monitoring of the study data as an on-going activity.

Staff involved in the study will ensure that all participants' confidentiality is maintained. The participants will be identified only by initials and surname (for purposes of address on the telephone), and a unique patient study identification number on the CRF and central electronic database. All documents will be stored securely and only accessible by staff involved in the study and authorised personnel. The study will comply with the Data Protection Act (in the UK) and with local legal requirements alongside the principles of ICH-GCP (worldwide); in line with this, all data will be anonymised as soon as it is practical to do so. The fully anonymised study data will be stored for at least five years following the closure of the study and thereafter disposed of in line with regulatory requirements. No participant will be individually identified in any subsequent publications relating to this study.

The study database will be kept on Microsoft Excel© 2016 and hosted on the University of Derby internal server. The server and database are protected by several measures including anti-virus and anti-spyware applications, firewalls, encryption technology and permissions. Data will be entered onto the paper CRF and retained and stored securely until the study end date, at which point the CRFs will be sent securely to the coordinating centre for entry onto the electronic study database. The data will be securely stored in line with ICH-GCP standards and data protection principles. The data stored will be checked for missing or unusual values and for consistency within participants over time. If any problems are identified, the appropriate CRFs will be reviewed in discussion with relevant local site personnel and queried for confirmation or correction as required until resolution.

The Chief and Principal Investigators, sponsor, and authorised staff will have access to participants' data. The Chief Investigator and/or Principal Investigators will facilitate access to study records for monitoring, audits, and regulatory inspections.

# 6.0 Data Retention and Archiving

In compliance with the ICH/GCP guidelines, regulations and following the University Hospitals NHS Trust Code of Research Conduct and Research Ethics the Chief Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 15 years (or for longer if required). If the Chief Investigator is no longer able to maintain the data records, an alternative person will be nominated to take over this responsibility.

The Trial Master File and Trial documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived by the sponsor local NHS Trust. This archive shall include all study databases and associated meta-data encryption codes.

### 6.1 Amendments

Amendments to the study protocol will be handled according to the Health Research Authority's guidelines for Non-CTIMP studies. Decisions to amend the protocol will be the responsibility of the Sponsor in consultation with the Chief Investigator and Principal Investigator. The Sponsor will have responsibility for determining the category of each amendment (Substantial versus Non-Substantial). All substantial and non-substantial amendments will be reported directly to the local Hospitals Trust Research Ethics Committee. It will be authorised by the Sponsor and Chief Investigator before submission. The appropriate documentation, clearly detailing changes, will be emailed to the responsible REC for review. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised participant consent forms and participant information sheets (if appropriate) have been reviewed and received approval from the REC and R&I departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the MHRA, R&I and REC are notified as soon as possible, and approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately and therefore the REC will be informed.

### 6.2 Access to the Final Study Dataset

The study database will be kept on Microsoft Excel© 2016 and hosted on the local University server. The server and database are protected by many measures including anti-virus and anti-spyware applications, firewalls, encryption technology and permissions. Data will be entered onto the paper CRF and retained and stored securely until the study end date, at which point the CRFs will be sent securely to the coordinating centre for entry onto the electronic study database.

The data will be securely stored in line with ICH-GCP standards and data protection principles. The data stored will be checked for missing or unusual values and for consistency within participants over time. If any problems are identified, the appropriate CRFs will be reviewed in discussion with relevant local site personnel and queried for confirmation or correction as required until resolution. The Chief and Principal Investigators and authorised staff will have access to participants' data. The Chief Investigator and/or Principal Investigators will facilitate access to study records for monitoring, audits, and regulatory inspections.

## 7.0 Financing and Insurance

This study is funded by GILEAD Sciences: COVID-19 RFP Program to the value of £174,885.46.

Indemnity and insurances will be provided by the University of Derby who is acting as the sponsor for this study.

### 7.1 Participant Incentives

There will be no financial recompense for the participants time, however all expenses (parking and travel) will also be covered by the study.

7.2 Funding

The study is funded by GILEAD Sciences: COVID-19 RFP Program

# 8.0 Public Patient Involvement

This protocol has been reviewed as part of the PPI groups at Royal Derby Hospital, UK and Patient involvement in Research Group (PIRG) at the Advanced Wellbeing Research Centre, UK.

# 9.0 End of Study

The end of the study is the date of the home visit (post-study follow up) of the last participant enrolled.

# 10.0 Discontinuation/Withdrawal of Participants from Study Treatment

Each participant has the right to withdraw from the study at any time. Participants may be withdrawn from the study either at their request or at the discretion of the Chief Investigator. Participants will be made aware (via The Participant Information Sheet and Participant Consent Form) that withdrawing the data which has been collected cannot be erased and may still be used in the final data analysis and study dissemination (i.e. published journal). The Chief Investigator may withdraw a participant from the study at any time if they consider that the participant's health is compromised by remaining in the study or the participant is not sufficiently cooperative. The reasons for any participant withdrawal will be recorded on the study completion form of the CRF. The data collected from withdrawn participants will be included in the study report.

Besides, the Chief Investigator may discontinue a participant from the study at any time if they consider it necessary for any reason including but not limited to:

- Ineligibility (arising during study or retrospective having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with study requirements
- An AE which results in the inability to continue to comply with study procedures
- Progression of illness that renders participants unable to comply with study procedures.
- Consent is withdrawn
- Lost to follow up

The reason for withdrawal will be clearly stated and recorded in the CRF.

If the participant is withdrawn due to an AE, the Chief Investigator will arrange for a follow-up telephone call until the AE has resolved or stabilised.

# 11.0 Dissemination

All data arising from this study will be owned by the named academics from the University of Derby. On completion of the study, the data will be analysed and reported into a final study report. The full study report will be accessible via the Chief Investigator.

There shall be no publication or dissemination of the conclusions of the study, including all or any part of the results of the study, without the prior written consent of the Chief Investigator and the Sponsor, such consent not to be unreasonably withheld or delayed. There shall be no publication or other dissemination of the conclusions of the study until the Sponsor has approved the conclusions of the study.

There are no plans to notify the participants of the outcome of the study. Participants may specifically request results from the PI; this information would be provided after study results have been published. The study protocol, full study report, anonymised participant-level dataset, and statistical code for generating the results will not be made publicly available.

Manuscripts resulting from the research will be conceived, written, and published at the discretion of the Chief Investigator, in conjunction with the Principal Investigator and other members of the research team as appropriate. This activity will be independent of the Research Funder, who will not have any control over the content or results of any publications. It is anticipated that the research will lead to publications in subject-specific international peer-reviewed journals and presentations at international conferences.

## 11.1 Authorship eligibility guidelines and any intended use of professional writers

Authorship will be determined by the research team on the final study report and subsequent publications, following the International Committee of Medical Journal Editors guidance for authorship of manuscripts submitted for publication. Professional writers will not be employed.

# 12.0 References

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