The PHIND study

Clinical evaluation of a POC assay to identify PHenotypes IN the Acute Respiratory Distress Syndrome

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Sponsor:	Queen's University Belfast 63 University Road Belfast BT7 1NN
Chief Investigator:	Prof Danny McAuley Professor/Consultant in Intensive Care Medicine Centre for Experimental Medicine 97 Lisburn Road, Belfast, BT9 7BL

PROTOCOL AUTHORISATION

Protocol Title	Clinical evaluation of a Point of Care (POC) assay to identify PHenotypes IN the Acute Respiratory Distress Syndrome
Protocol Acronym (if applicable)	PHIND
Protocol Number	B19/06
Protocol Version Number/Date	v6.0_Final_29.05.20
Protocol Amendments	Amendment 6 (substantial) moving from Protocol v5.0_Final_11.12.19 Amendment 2 (substantial) moving from Protocol v4.0_Final_10.09.19. Amendment 1 (Minor) moving from protocol_v3.0_Final_07.05.19. Protocol v1.0 and v2.0 were not implemented.

A review of the protocol has been completed and is understood and approved by the following:

Danny McAuley	_____	29 / 05 / 2020
Chief Investigator Name	Signature	Date
Clíona McDowell	_____	29 / 05 / 2020
Statistician Name	Signature	Date

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LIST OF ABBREVIATIONS

Abbreviation / Acronym	Full Wording
APACHE	Acute Physiology and Chronic Health Evaluation
ARDS	Acute Respiratory Distress Syndrome
CI	Chief Investigator
CMP	Case Mix Programme
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
CTIMP	Clinical Study of an Investigative Medicinal Product
CTU	Clinical Trials Unit
DNAR	Do Not Attempt Resuscitation
ECMO	Extracorporeal Membrane Oxygenation
$ECCO_2R$	Extracorporeal CO_2 Removal
GCP	Good Clinical Practice
GP	General Practitioner
HSE	Health Service Executive
ICH	International Conference on Harmonisation
ICNARC	Intensive Care National Audit & Research Centre
ICU	Intensive Care Unit
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Study Number
HFNO	High Flow Nasal Oxygen
LPS	Lipopolysaccharide
MDM	Monocyte-derived Macrophages
MDT	Multi-Disciplinary Team
NHS	National Health Service
NICTU	Northern Ireland Clinical Trials Unit
NPV	Negative Predictive Value
PBW	Predictive Body Weight
PBMC	Peripheral Blood Mononuclear Cells
PEEP	Positive end-expiratory Pressure
PI	Principal Investigator
POC	Point of Care
QUB	Queen's University Belfast
REC	Research Ethics Committee
RR	Respiratory Rate
RMP	Registered Medical Practitioner
ROI	Republic of Ireland
SAP	Statistical Analysis Plan
SOFA	Sequential Organ Failure Assessment
SOP	Standard Operating Procedure
TMF	Trial Master File
TMG	Trial Management Group
SSC	Study Steering Committee
UK	United Kingdom
VFD	Ventilator Free Day

1 STUDY SUMMARY

Protocol Title	Clinical evaluation of a Point of Care (POC) assay to identify PHenotypes IN the Acute Respiratory Distress Syndrome
Health condition(s) or problem(s) studied	Acute Respiratory Distress Syndrome (ARDS)
Study Design	A multi-centre, prospective, observational study
Study Aim and Objectives	**Aim** The purpose of this project is to prospectively define hyper- and hypo-inflammatory phenotypes in patients with ARDS and determine clinical outcomes associated with each phenotype. **Objectives**: The main objectives of this study are to: Primary: (i) Assess the clinical outcomes in patients with ARDS according to their prospectively defined inflammatory phenotype determined using a POC assay Secondary: (i) Assess the agreement of the phenotype allocation using a POC assay and the clinical study dataset (ii) Assess the stability of phenotype allocation over time (iii) Feasibility of delivering a POC assay in the NHS intensive care setting Results of group allocation will be blinded to clinical and research staff.
Primary Outcome	Mortality at 60 days in the hyper-inflammatory and hypo-inflammatory phenotypes in patients with ARDS

Secondary Outcomes	Secondary outcome measures of the study are: (i) Difference in other important clinical outcomes (ii) Agreement of phenotype classification using a POC assay and standard laboratory based assays (iii) Agreement of phenotype classification using a POC assay and the clinical study dataset (iv) Agreement of phenotype classification between day 1 and 3 (v) Feasibility as measured by POC device performance
Inclusion and Exclusion Criteria	**Inclusion criteria**: 1. Patient receiving mechanical ventilation, CPAP or high flow nasal oxygen (HFNO) 2. ARDS as defined by the Berlin definition. **Exclusion criteria**: 1. Age <18 years of age 2. More than 48 hours after onset of ARDS 3. Receiving ECMO at the time of recruitment 4. Treatment withdrawal imminent within 24 hours 5. DNAR (Do Not Attempt Resuscitation) order (excluding advance directives) in place 6. Declined consent 7. Prisoners
Countries of Recruitment	United Kingdom and Republic of Ireland
Study Setting	Adult Intensive Care Units (ICU)
Target Sample Size	480. An additional cohort of patients with COVID-19 will be recruited.
Study Duration	At least 24 months

2 STUDY TEAM

Chief Investigator	**Prof Danny McAuley**
Co-Investigators	**Prof Cecilia O'Kane** Queen's University Belfast
	Dr Murali Shyamsundar Queen's University Belfast
	Prof Anthony Gordon Imperial College London
	Dr Manu-Shankar Hari Kings College London
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	Dr Matthieu Jabaudon Université Clermont Auvergne
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3. BACKGROUND AND RATIONALE

3.1 Background Information

3.1.1 Acute respiratory distress syndrome is a common and serious condition with no specific therapy

Acute respiratory distress syndrome (ARDS) is an inflammatory condition that results in severe respiratory failure and the need for mechanical ventilation. It is a syndrome with significant global burden and accounts for approximately 24% of mechanically ventilated patients in intensive care units [1]. It is estimated to account for approximately 75000 deaths annually in the USA alone [2].

Despite decades of research, mortality due to ARDS remains high at 35–46% [1], with increasing mortality in patients with more severe lung injury. ARDS survivors have significant long term comorbidity [3] with reduced quality of life even 5 years after disease resolution [4]. Various pharmacological agents such as β2 agonists, statins, keratinocyte growth factor and aspirin [5-9] have been investigated as potential therapies to prevent or treat ARDS, however to date there is no effective pharmacological therapy for ARDS and current treatment strategy is largely supportive [10].

One reason for the lack of specific pharmacological therapy is likely due to the clinical and biological heterogeneity. It is essential to rapidly identify patients with specific therapy responsive traits to improve our chance of identifying a specific therapy.

3.2 Rationale for the Study

3.2.1 ARDS phenotypes have different outcomes and response to therapy

The clinical and biological heterogeneity in ARDS makes it essential to identify homogenous phenotypes when investigating potential therapies.

A retrospective analysis of the clinical and biological data-set collected as part of two large multicentre studies (ARMA and ALVEOLI) using latent class analysis has identified at least two ARDS phenotypes [11]. Furthermore these two phenotypes could be differentiated using a parsimonious data-set including the presence of shock, metabolic acidosis and a higher inflammatory status (IL-6 and sTNFr1). The hyper-inflammatory phenotype demonstrated significantly worse outcomes when compared to the hypo-inflammatory phenotype with higher mortality and less ventilator free and organ failure free days. In the ALVEOLI study, where low PEEP was compared to high PEEP strategy, the two phenotypes demonstrated a differential response to PEEP suggesting the potential for using this phenotypic classification in identifying a therapy responsive trait.

In addition, in a secondary analysis of the HARP-2 study, a multicentre study investigating the potential of simvastatin as an anti-inflammatory therapy for ARDS, the presence of a hyper- and hypo-inflammatory phenotype was confirmed. The hyper-inflammatory phenotype had a higher 28 day mortality, fewer ventilator free days and

organ failure free days. Survival of patients classified as hyper-inflammatory and randomised to simvastatin was improved [12].

Implementation of a precision medicine approach to identify patients with a therapy response trait is crucial to identify specific therapies to prevent or treat ARDS. Development of a Point of Care (POC) assay for IL-6 and sTNFr1 for prospective confirmation of the inflammatory phenotypes using the parsimonious data-set in patients with ARDS will support a precision medicine approach for this condition.

ARDS occurs in approximately 20% of COVID-19 cases and respiratory failure is the leading cause of mortality [13]. In a retrospective multi-centre study of 150 confirmed cases in Wuhan, China, ARDS occurred in a greater proportion of non-survivors, 81% (55/68 patients) compared with only 9% of survivors (7/82 patients) [13]. In another study of 193 confirmed COVID-19 cases, ARDS was observed at a significantly higher rate in non-survivors [14]. Therefore the opportunity to recruit patients with COVID-19 presents an opportunity to understand the prevalence of inflammatory phenotypes in ARDS due to COVID-19.

3.2.2 A POC assay will support precision medicine for ARDS

Studies that show no benefit from an intervention could occur as a result of a variety of reasons including a) the intervention was ineffective, b) the study design was poor or c) patient heterogeneity. Reduction of patient heterogeneity to identify patients with common biological processes will enable the selection of patients with a higher likelihood of therapy response in clinical studies. The identification and institution of therapy for critically ill patients with ARDS needs to occur rapidly in view of the nature of the disease and development of an accurate POC assay is likely to be an essential component in the discovery of effective therapies.

4. STUDY AIMS AND OBJECTIVES

4.1 Research Hypothesis

Patients prospectively classified to the hyper-inflammatory ARDS phenotype on the basis of clinical characteristics and a novel POC biomarker assay will have worse clinical outcomes than the hypo-inflammatory phenotype.

4.2 Study Aim

The purpose of this project is to prospectively identify hyper- and hypo-inflammatory phenotypes in patients with ARDS and determine clinical outcomes associated with each phenotype.

4.3 Study Objectives

4.3.1 Primary Objective

The primary objective of this study is to assess the clinical outcomes in patients with ARDS according to their prospectively defined inflammatory phenotype determined using a POC assay.

Results of group allocation will be blinded to clinical and research staff.

4.3.2 Secondary Objectives

The secondary objectives of this study are to:

(i) Assess the agreement of the phenotype allocation using the POC assay and the clinical study dataset [11].
(ii) Assess the stability of phenotype allocation over time
(iii) To test feasibility of delivering a POC assay in the NHS/HSE intensive care setting.

5. OUTCOME MEASURES

5.1 Primary Outcome Measure

The primary outcome is mortality at 60 days in the hyper-inflammatory and hypo-inflammatory phenotypes in patients with ARDS.

5.2 Secondary Outcome Measures

Secondary outcomes are:

(i) Difference in time to extubation, intubation rate in patients on HFNO, re-intubation rate, ventilator free days at day 28, duration of ventilation, length of intensive care unit (ICU) and hospital stay as well as 28-day mortality.
(ii) Agreement of phenotype classification using a POC assay and standard laboratory based assays
(iii) Agreement of phenotype classification using a POC assay and the clinical study dataset.
(iv) Agreement of phenotype classification between day 1 and 3
(v) Feasibility of delivering a POC assay in NHS/HSE intensive care setting as measured by assay technical failure rate.

5.3 Exploratory Analyses

Measurements will include:

1. Systemic inflammatory responses will be assessed by the following:
   Plasma and serum inflammatory response biomarkers which may include but are not limited to measurement of plasma CRP, cytokines (including but not limited to TNFα, sTNFR1 and 2, IL-1β, IL-6, IL-8, IL-10), lipocalins, proteases and antiproteases, adhesion and activation molecule expression (including but not limited to sICAM1), NETs, coagulation factors (including but not limited to thrombin-antithrombin complex, tissue factor, protein C, thrombomodulin and plasminogen activator inhibitor, activated FVII-antithrombin complex, FXI-AT, FXII-AT, C3a and sC5b-9, d-dimer and fibrinogen), circulating miRNAs, extracellular vesicles and RAGE ligands will be undertaken. Specific cellular populations within the blood (using but not limited to cytospins and flow cytometry) and identification of transcriptome changes within these cell populations will be investigated. Genetic markers predisposing to the hyper and hypo inflammatory phenotypes will be measured.

2. Indices of pulmonary and systemic epithelial and endothelial function and injury will be assessed by the following:
   Plasma and serum biomarkers which may include but not limited to measurement of RAGE, Ang I/II, SP-D, vWF, PCP3 as well as total protein, plasma albumin, α2-macroglobulin, and protein permeability (albumin:α2-macroglobulin ratio) will be undertaken. Urinary albumin/creatinine ratio and makers of extracellular matrix degradation including but not limited to desmosine will also be measured.

3. Samples from subjects may also be tested on primary cultures of fresh human neutrophils monocytes and macrophages as well as mesenchymal stromal cells to provide mechanistic insights. Measurements may include but will not be limited to the measurement of cell activation (shape change, CD11b surface expression, superoxide release), adhesion and transmigration, mitochondrial activity, cytokine release and MMP production, rate of apoptosis and their ability to phagocytose.

4. Monocytes or neutrophils will be isolated from blood. Cells will be stimulated or monocytes matured for 5-7 days to produce monocyte-derived macrophages (MDMs) prior to stimulation. Cells will be stimulated with LPS or other inflammatory stimuli to identify mechanisms modulating inflammatory responses in these cells during ARDS.
   Human peripheral blood mononuclear cells (PBMC) will be isolated from blood using density centrifugation and stored with freezing medium in nitrogen tanks until use. Cryopreserved PBMCs will be thawed in standard media. Immune profiling of PBMCs based on the associations observed in the primary outcome and stability of the phenotypes will be undertaken.

5. Cardiac function as determined by echocardiography (ECHO data will only be collected if available clinically).

If measurements for exploratory analyses cannot be undertaken, this will not be recorded as a protocol deviation.

None of these additional exploratory analyses will be performed using the POC assay.

6. STUDY DESIGN

6.1 Study Design

This is a multi-centre, prospective, observational study.

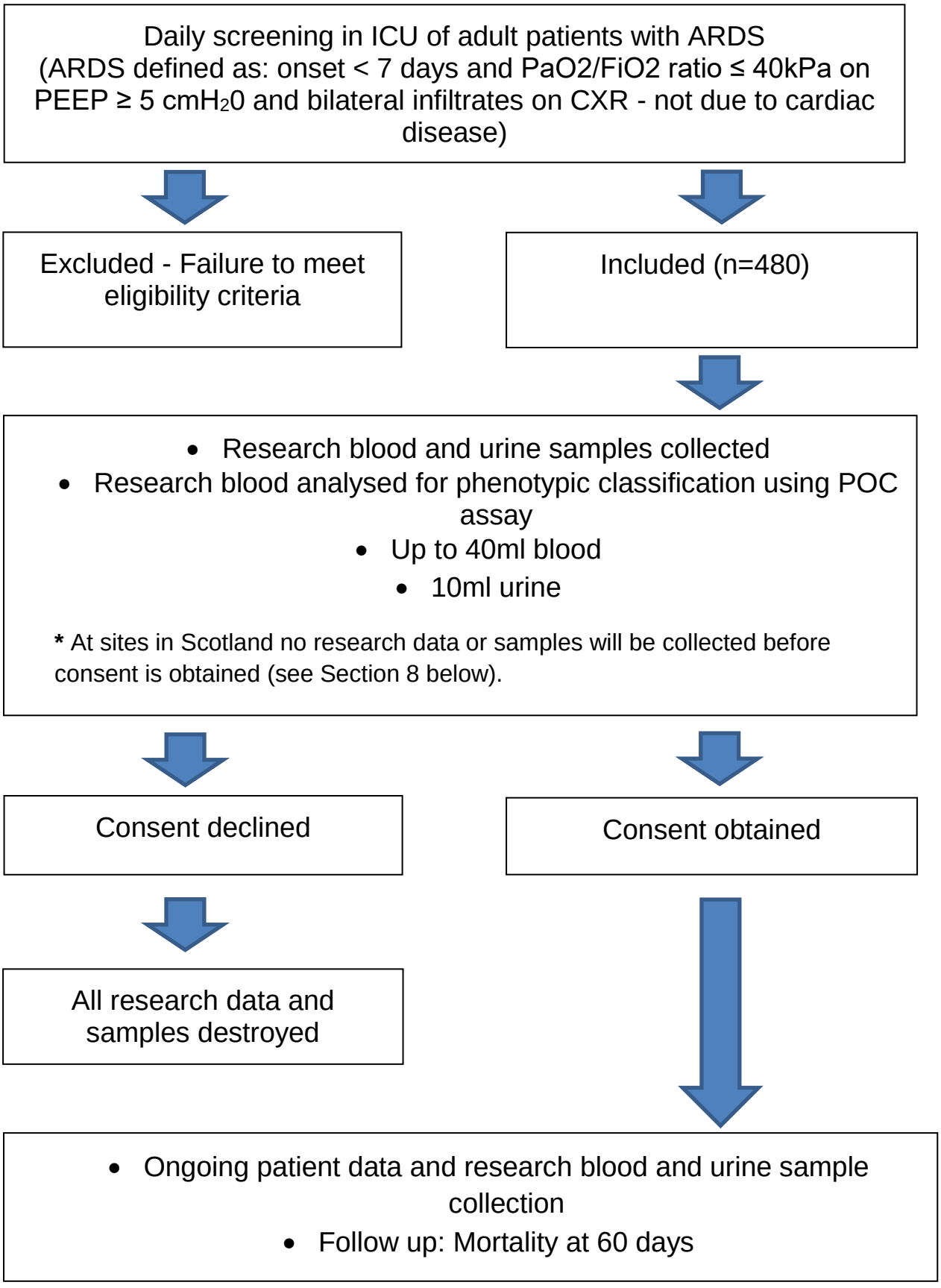
6.2 Study Setting

At least 22 adult ICUs will participate.

6.3 Study Schematic Diagram

Figure 1

PHIND Schematic Diagram



6.4 Study Timeline

It is planned that the recruitment period will start in November 2019 and continue for at least 18 months. Following the completion of recruitment and follow-up, there will be a close out period.

6.5 End of Study

For the purposes of submitting the end of study notification to the Sponsor and Research Ethics Committee (REC) the end of study will be considered to be when database lock occurs for the final analysis. The study will be stopped prematurely if:

- Mandated by the Research Ethics Committee (REC)
- Mandated by the Sponsor (e.g. following recommendations from the Study Steering Committee (SSC)
- Funding for the study ceases

The REC originally providing a favourable opinion of the study will be notified in writing once the study has been concluded or if terminated early.

7. PATIENT ELIGIBILITY, SCREENING AND RECRUITMENT

7.1 Eligibility Criteria

Patients will be screened for eligibility based on the inclusion/exclusion criteria outlined below. Eligibility to participate in the study will be confirmed by a medically qualified person.

7.1.1 Inclusion criteria

1. Patient is receiving mechanical ventilation, CPAP or high flow nasal oxygen (HFNO)
2. ARDS as defined by the Berlin definition [15]
   a) Onset within 1 week of identified insult
   b) Within the same 24-hour time period:
      i. Hypoxic respiratory failure (PaO2/ FiO2 ratio ≤ 40kPa on PEEP ≥ 5 cmH20*)
      ii. Bilateral infiltrates consistent with pulmonary oedema not explained by another pulmonary pathology
      iii. Respiratory failure not fully explained by cardiac failure or fluid overload

The time of onset of ARDS is when the last ARDS criterion is met.
*PEEP assumed ≥ 5 cmH20 if on HFNO

7.1.2 Exclusion criteria

1. Age <18 years of age
2. More than 48 hrs after onset of ARDS
3. Receiving ECMO at the time of recruitment
4. Treatment withdrawal imminent within 24 hours
5. DNAR (Do Not Attempt Resuscitation) order (excluding advance directives) in place
6. Declined consent
7. Prisoners

7.2 Co-enrolment Guidelines

Patients in the PHIND study are potentially eligible for co-enrolment in other studies, this will be decided on a case by case basis in keeping with UK guidelines for critical care research [16]. Co-enrolment with any studies should be documented in the Case Report Form (CRF).

7.3 Screening Procedure

All mechanically ventilated patients in the ICU will be screened daily each morning for eligibility.

All screening data must be recorded by the Principal Investigator (PI) or designee to document all patients screened for the study and all patients recruited. Patients screened and not recruited on to the study will be documented, including the reason(s)

for not being enrolled on the study. The PI or designee will be required to submit screening data to the Clinical Trials Unit (CTU) approximately every month.

8. **CONSENT**

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. The PI (or designee) is responsible for ensuring that the requirements for study participation are in place. This can be obtained by an appropriately trained doctor or nurse, who must be Good Clinical Practice (GCP) trained, suitably qualified and experienced and have been delegated this duty on the delegation log. Appropriate signatures and dates must be obtained on the required documentation.

The incapacitated nature of patients in ICU due to, for example, the effects of critical illness, sedation, and delirium will usually preclude obtaining prospective informed consent from participants. For patients who lack capacity to give informed consent the legal requirements to recruit patients without capacity will be followed. The regulations for the recruitment of patients lacking capacity differs across the legal jurisdictions in which the participating sites in this study are based. Practice will adhere to local regulations in England and Wales (Mental Capacity Act 2005), Northern Ireland (Mental Capacity Act 2016), Scotland (Adults with Incapacity (Scotland) Act 2000) and in ROI (The Assisted Decision Making (Capacity) Act 2015) and the Health Research Regulations 2018.

If the POC assay is to prove to be effective, it will need to be undertaken as it would be applied in real life i.e. as soon as possible after a patient develops ARDS, to enable personalised treatment to be commenced. The result at ARDS diagnosis may differ from a sample taken later. The other research samples need to be collected at the same time, as a secondary objective is to confirm the results of the POC assay with laboratory based assays and therefore these samples must be taken at the same time for accurate comparisons.

So, given the very low risk associated with blood sampling and that there will be no change to standard care, to avoid delay, a deferred consent/assent process will be applied in England, Wales, Northern Ireland and ROI, subject to local laws and ethical review processes. Samples will initially be collected as outlined in section 9 of this protocol and will be held at site for up to 7 days pending agreement for the patient's participation in the study. The samples will be analysed for the phenotypic classification on the POC assay and initial clinical data will be collected. In the event that agreement for patient participation is not obtained or the regulations for the recruitment of patients lacking capacity cannot be met by day 7, the data will not be used and the samples will be destroyed. No samples will be transferred to the QUB laboratory without agreement for patient participation having first been obtained (see Section 8 below).

In Scotland (where deferred consent is not allowed for non-CTIMP studies), telephone consent will be sought if no Nearest Relative/Welfare Guardian or Welfare Attorney is at the site when eligibility is confirmed (see Section 8.2 below).

8.1 England, Wales and Northern Ireland

The researcher will seek advice from a Personal Consultee (who may be a relative, partner or friend of the participant). This should normally take place during a face-to face meeting. An authorised staff member/researcher will describe the study to the individual, and provide them with a Covering Statement, Information Sheet and Personal Consultee Declaration form (England/Wales and Northern Ireland). The researcher will seek their views about whether the patient should take part in the study. They will be asked about their opinion of the wishes and feelings of the patient if they had capacity.

After the researcher has checked that the information sheet is understood, the researcher will invite the Personal Consultee to sign the form and will then countersign it. The original will be retained in the investigator site file (ISF) and a copy given to the person who signed the form and another copy placed in the patients' medical records.

If the Personal Consultee is not available at site, the researcher may contact the Personal Consultee by telephone and seek verbal agreement. This verbal agreement will be recorded in the Consultee Telephone Agreement Form. The Consultee Telephone Agreement Form will be signed by a second member of staff who has witnessed the telephone advice. This witness may be a member of the site study team or site clinical staff.

The original Consultee Telephone Agreement Form will be retained in the ISF and a copy placed in the patients' medical records. Written agreement will then be obtained as soon as possible. Where telephone agreement is in place, but a patient dies before written agreement can be obtained, then the verbal agreement will remain valid.

In the event that there is no Personal Consultee for sites in England, Wales and Northern Ireland authorisation to recruit the patient will be sought from a Registered Medical Practitioner (RMP) who should be a doctor unrelated to the study conduct. The RMP will be informed about the study by a member of the research team and given a copy of the Registered Medical Practitioner Form (England/Wales and Northern Ireland) and a copy of the Covering Statement, Information Sheet and Personal Consultee Declaration (England/Wales and Northern Ireland).

If the RMP decides that the patient is suitable for entry into the study, they will be asked to complete the relevant authorisation form. The original will be retained in the ISF and a copy given to the person who signed the form and another copy placed in the patients' medical records. In the event that a Personal Consultee is identified after RMP advice is obtained, the above process for Personal Consultee Declaration will be followed and all forms will be filed as instructed above.

8.2 Scotland

The researcher will seek permission from a Welfare Attorney, Welfare Guardian or Nearest Relative. If the patient's representative is available at the site when eligibility is confirmed, permission will be sought during a face-to-face meeting. An authorised staff member/researcher will describe the study to the individual, and provide them with a Covering Statement, Information Sheet and Consent Form for the Welfare Attorney, Welfare Guardian or Nearest Relative (Scotland). The researcher will seek

their views about whether the patient should take part in the study. They will be asked to give their consent based on their opinion of the wishes and feelings of the patient if they had capacity. After the researcher has checked that the information sheet is understood, the researcher will invite the Welfare Attorney, Welfare Guardian or Nearest Relative to sign the form and will then countersign it. The original will be retained in the ISF and a copy given to the person who signed the form and another copy placed in the patients' medical records.

If there is no Welfare Attorney, Welfare Guardian or Nearest relative available at site when eligibility is confirmed, the researcher may contact the Welfare Attorney, Welfare Guardian or Nearest relative by telephone and seek verbal agreement. This verbal agreement will be recorded in the Consultee Telephone Agreement Form. The Consultee Telephone Agreement Form will be signed by a second member of staff who has witnessed the telephone agreement. This witness may be a member of the site study team or site clinical staff. The original Consultee Telephone Agreement Form will be retained in the ISF and a copy placed in the patients' medical records. Written agreement will then be obtained as soon as possible. Where telephone agreement is in place, but a patient dies before written agreement can be obtained, then the verbal agreement will remain valid.

8.3 Republic of Ireland

Assent will be obtained if possible from the patient's Next of Kin. This should take place during a face-to face meeting. An authorised staff member/researcher will describe the study to the individual, and provide them with a Research Study Information and Assent Form. The researcher will seek their views about whether the patient should take part in the study. They will be asked to give their assent based on their opinion of the wishes and feelings of the patient if they had capacity. After the researcher has checked that the information sheet is understood, the researcher will invite the Next of Kin to sign the form and will then countersign it. The original will be retained in the ISF and a copy given to the person who signed the form and another copy placed in the patients' medical records.

Assent cannot be obtained by telephone or by a Registered Medical Practitioner unrelated to the study.

8.4 Patient Consent to Continue

Once the participant has recovered from the condition / treatment causing incapacity they will be approached to obtain permission to continue in the study. Patients will be informed of their participation in the study by the responsible clinician or a member of the research team once they regain capacity to understand the details of the study. The responsible clinician or a member of the research team will discuss the study with the patient and the patient will be given a copy of the Patient Information Sheet and Consent Form for Participants with Recovered Capacity or the Participant Information and Consent to Continue Form. In all regions patients will be asked for consent to participate in the study and to sign the Consent to Continue Form which will then be countersigned by the person taking consent. The original will be retained in the ISF and a copy given to the patient and another copy placed in the patients' medical records. In the rare event that consent to continue cannot be obtained, the agreement to recruit the patient will remain valid.

If the participant declines on-going participation, only anonymised data recorded up to the point of withdrawal will be included in the study analysis.

8.5 Withdrawal from the Study

Participants may be withdrawn from the study at any time without prejudice.

If patient consent is withdrawn or a patient's representative states enrolment in the study would be against the patient's wishes or best interests; the option to withdraw from the study will be given and documented in the patient's notes and in the CRF.

If the patient or patient representative declines on-going participation, anonymised data recorded up to the point of withdrawal will be included in the study analysis unless the patient or patient representative requests otherwise. We will seek their permission to continue to collect patient data during the patient's hospital admission and to follow up to ascertain the patient's long term health status using NHS/HSC/HSE or other hospital information systems, NHS Digital or via the patient's GP (if the patient has been discharged). In the event that the patient or patient representative withdraws consent for the long term storage of blood samples, any stored samples will be destroyed.

9. SCHEDULE OF ASSESSMENTS AND STUDY PROCEDURES

9.1 Schedule of Assessments

All patients will be evaluated during the study and data collected at each of the following time-points. For routinely collected clinical data the National Health Service (NHS) or Health Service Executive (HSE) record will be the source document and for study specific clinical measurements the CRF will be the source document. Recruitment is defined as time of blood sample collection for the baseline POC assay.

Day 0 (Baseline)

Baseline data (day 0) is the 24 hours preceding the time of recruitment. If more than one value is available for this 24-hour period the value closest but prior to the time of recruitment will be recorded unless specified. Day 0 (baseline) data collected will include but is not limited to:

- Patient demographics
- Date of birth, gender, height, weight, race, PBW
- Smoking history/E-cigarette use/alcohol use
- Co-morbidity
- ICNARC Case Mix Programme (CMP) number or equivalent
- Date/ time of ICU admission
- Date/time of onset of mechanical ventilation
- Date/time of agreement/consent and recruitment
- Aetiology of ARDS
- The Acute Physiology And Chronic Health Evaluation score II (APACHE II)
- Vital signs including but not limited to: temperature, heart rate, and systolic blood pressure
- Haematology data including but not limited to: haematocrit and white cell count
- Biochemistry data including but not limited to: sodium, glucose and albumin
- First qualifying P/F ratio (including date/time)
- Worst P/F ratio (including date/time)
- Murray Lung Injury Score
- Determinants of the sequential organ failure assessment (SOFA) score
- Ventilation parameters including but not limited to: Mode of ventilation, minute volume, RR, mean airway pressure, plateau pressure, PEEP
- Arterial blood gas including but not limited to FiO2, PaO2, PaCO2, pH, lactate and bicarbonate
- Oxygenation Index
- Use of adjunctive therapies
- Steroids (name/dose), statin (name/dose), insulin and nutritional intake.
- Child Pugh Score in patients with known chronic liver disease
- Renal replacement therapy
- Research blood and urine sample collected
- Clinical or PCR diagnosis of COVID-19 where appropriate

Day 1

Day 1 is the remainder of the calendar day from the time of recruitment to midnight. Thereafter subsequent days are calendar days.

- POC assay data
- POC assay technical issues (need to repeat assay)
- POC device failure
- POC assay coefficient of variation (where available)
- Time from sample acquisition to POC assay completion

Day 3, 7, 14 and 28

All measurements will be recorded and collected between 6 - 10am or as close to this time as possible, unless otherwise stated in the CRF. Data will be collected where available to ICU discharge. Data will include but is not limited to:

- Vital signs including but not limited to: temperature, heart rate, and systolic blood pressure (day 3 only)
- Haematology data including but not limited to: haematocrit and white cell count (day 3 only)
- Biochemistry data including but not limited to: sodium, glucose and albumin (day 3 only)
- Ventilation parameters including but not limited to: Mode of ventilation, minute volume, RR, mean airway pressure, plateau pressure, PEEP
- Arterial blood gas including but not limited to FiO2, PaO2, PaCO2, pH, lactate and bicarbonate
- Use of adjunctive therapies
- ECHO data if available (data within 7 days following recruitment can be used)
- Steroids (name/dose), statin (name/dose), insulin and nutritional intake
- Renal replacement therapy
- Overall fluid balance for preceding day (based on local practice for defining 24 hour fluid balance)
- Research blood and urine sample collected (day 3 only)
- POC assay data (day 3 only)
- POC assay technical issues (need to repeat assay) (day 3 only)
- POC device failure (day 3 only)
- Time from sample acquisition to POC assay completion (day 3 only)

The following data will also be collected:

- Date/time of intubation if on HFNO
- Date/time of extubation
- Date/time of re-intubation (excludes ‘reinsertion for procedure only’ i.e. temporary elective reintubations)
- Date/time of discontinuation of mechanical ventilation (unassisted breathing)
- Date/time of critical care discharge
- Date/time of hospital discharge
- Date/time of death, including cause of death

Extubation is defined as first time being successfully free from an endotracheal tube or a tracheostomy tube for 48 hours.

Unassisted breathing i.e. no ventilatory support is defined as; extubated with supplemental oxygen or room air, or open T-tube breathing, or tracheostomy mask breathing, or CPAP without inspiratory pressure support for 48 hours. Patients receiving pressure support via non-invasive ventilation (except for sleep disordered breathing) or extra-corporeal lung support will be defined as receiving ventilatory support.

Discharge from critical care is defined as first discharge to a ward in the hospital or another hospital; a transfer between ICUs is not considered a discharge from critical care. Hospital discharge is the first date that the patient is discharged to home/community, a transfer between hospitals is not considered as a hospital discharge.

Ventilator free days (VFDs) to day 28 are defined as the number of days from the time of initiating unassisted breathing to day 28 after the date of commencing ventilatory support, assuming survival for at least 48 hours after initiating unassisted breathing and continued unassisted breathing to day 28. If the patient was receiving ventilatory support prior to ICU admission, the ICU admission date will be considered as the start of the VFD calculation. If a patient returns to assisted breathing and subsequently achieves unassisted breathing to day 28, VFDs will be counted from the end of the last period of assisted breathing to day 28. A period of assisted breathing lasting less than 24 hours and for the purpose of a surgical procedure will not count against the VFD calculation. If a patient was receiving assisted breathing at day 27 or dies prior to day 28, VFDs will be zero. Patients transferred to another hospital or other health care facility will be followed to day 28 to assess this endpoint.

Time to extubation will be counted from date of intubation to extubation, or from the date of ICU admission to successful extubation, if the patient was intubated prior to ICU admission.

Duration of ventilation will be counted from the date of commencing ventilatory support, or the date of ICU admission, if the patient was receiving ventilatory support prior to ICU admission, to the time of initiating unassisted breathing.

Duration of critical care and hospital stay will be counted from ICU admission to discharge.

9.2 Study Procedures

9.2.1 Blood and Urine Sampling

The following samples will be collected by trained staff and managed according to the Sample Processing Guideline:

Blood

Baseline – up to 40ml
Day 3 - up to 40ml

Urine

Baseline – 10ml
Day 3 – 10ml

The blood sample will be measured on the POC assay using an Evidence MultiSTAT Analyser as soon as possible after collection and ideally within 10 minutes. Samples will be analysed by staff trained in the use of the POC assay. In a subset of patients where possible we will measure the POC assay in duplicate to determine the coefficient of variation. Where the POC assay is undertaken in duplicate, the first POC assay will determine the group allocation. Appropriate QC and QA of the Evidence MultiSTAT Analyser and POC assay will be undertaken periodically.

The remaining blood and urine samples will be labelled with a unique Participant Study Number. After processing locally, samples will be transferred to QUB. Samples will be stored at -80^0C until further analysis. If the baseline POC assay is not collected this will be recorded as a protocol deviation, however if other research blood and urine samples cannot be collected this will not be recorded as a protocol deviation.

Samples will be stored beyond study completion in QUB. As new scientific data become available we will be able to use this resource of stored samples to investigate these new data pending additional ethical approval if required.

9.2.2 Phenotype Allocation Using the POC Assay

Data from the POC assay will be entered into the study database, along with the clinical data needed to classify phenotype (plasma bicarbonate). The database will then automatically generate a phenotype allocation using a parsimonious model developed by Drs. Sinha, Delucchi and Calfee (Sinha et al, ATS 2018 and manuscript in preparation). This model has been derived using data from 2022 patients enrolled in the ARMA, ALVEOLI and FACTT studies, using comparison to phenotype as assigned by latent class analysis. Model coefficients have been fixed, and a probability cut-off of 0.5 will be used to assign phenotype. This model, and the probability cut-off has been validated in independent databases SAILS and HARP-2 clinical trials; Sinha P et al, under submission) and shown to perform well in those datasets. Phenotype allocation will be prospectively defined.

9.2.3 Participant Follow Up

Data will be censored at 60 days for all outcomes from the date of last patient recruited. Patient survival after discharge from hospital will be determined either from hospital/regional information systems (e.g. electronic care record) or by using NHS Digital if available in that region or by contacting the GP (which will be undertaken centrally by Northern Ireland Clinical Trials Unit (NICTU) staff).

9.2.4 Clinical Management of Patients in the Study

There will be no change to standard care treatment.

10. DATA COLLECTION & MANAGEMENT

10.1 Data Quality

Data integrity and study credibility depend on factors such as ensuring adherence to the protocol and using quality control measures to establish and maintain high standards for data quality.

The Chief Investigator (CI) and CTU will provide training to site staff on study processes and procedures including the CRF and data collection.

Quality control is implemented by the CTU in the form of Standard Operating Procedures (SOPs), which are defined to encompass aspects of the clinical data management process, and to ensure standardisation and adherence to International Conference of Harmonisation Good Clinical Practice (ICH-GCP) guidelines and regulatory requirements.

Data validation will be implemented and discrepancy reports will be generated following data entry to identify discrepancies such as out of range, inconsistencies or protocol deviations based on data validation checks programmed in the clinical study database.

10.2 Data Collection

All data for an individual patient will be collected by the PI or designee and recorded in source documents/electronic CRF for the study. Patient identification on the CRF will be through their unique study identifier, allocated at the time of recruitment.

Data should be entered onto the online electronic study database as per the CRF entry guidelines.

10.3 Data Management

Following the entry of patient data into the study database, the data will be processed as per the CTU SOPs. Data queries will be generated for site staff as required to clarify data or request missing information. The designated site staff will be required to respond to these queries within approximately 2 weeks. All queries will be responded to and resolved within the study database. Any amended information will then be entered in the study database.

11. STATISTICAL CONSIDERATIONS

11.1 Sample Size

Data from several retrospective analyses have shown that the percentage of patients with ARDS in the hyper-inflammatory sub-group was 27-34% of the overall population, that the mortality in the hypo-inflammatory sub-group was 17-23%, with a mortality difference between the hyper-inflammatory and hypo-inflammatory sub-groups ranging from 15-32%. A conservative percentage of patients in the hyper-inflammatory sub-group of 25%, a mortality in the hypo-inflammatory sub-group of 17% and a difference in 28-day mortality between the hyper-inflammatory and hypo-inflammatory sub-groups of 15% was assumed. The mortality in the hyper-inflammatory group is assumed to be 17% under the null hypothesis and 32% under the alternative hypothesis [11, 12, 17, 18].

Based on a two group chi-square test, group sizes of 347 in the hypo-inflammatory group and 116 in the hyper-inflammatory group achieves 90% power at a two-sided significance level of 0.05 to detect a mortality difference between the groups of 15%. Based on our previous experience the group sizes have been inflated by 3% to allow for technical failure, drop-out or loss to follow-up giving group sizes of 360 in the hypo-inflammatory group and 120 in the hyper-inflammatory group.

An additional exploratory cohort of patients with COVID-19 will be recruited. No formal sample size calculation for this cohort is feasible. An indicative sample of up to 100 patients will be recruited in this cohort during the course of the study, however it is recognized that this is an estimate which will be dependent on the duration of the COVID-19 pandemic.

11.2 Statistical Methods

A detailed Statistical Analysis Plan (SAP) will be written for the main study by the study Statistician and approved by the SSC and made openly available prior to any study analysis being undertaken.

Baseline characteristics, and follow-up measurements will be described using the appropriate descriptive summary measures depending on the scale of measurement. For the primary outcome and other dichotomous outcomes, risk ratios and 95% confidence interval (CI) will be calculated. The primary outcome of 60 day mortality will be analysed using chi-square and a secondary analysis using logistic regression to adjust for variables known to be clinically important in determining outcome such as age, co-morbidity, aetiology of ARDS, vasopressor requirement and PF ratio will also be carried out. The comparison of continuous outcomes between the two groups will be investigated using analysis of covariance, adjusting for other covariates where appropriate. Time-to-event outcomes will be analysed by survival methods and reported as hazard ratios with 95% CI.

The primary analysis will be using the phenotype allocation defined by the POC assay. Secondary analyses will use the phenotype allocation defined by the standard laboratory assays and allocation defined by the complete data-set using latent class

analysis. Agreement between phenotype allocation, using these 3 methods will be compared using, McNemar's test.

Subsequent analyses after study completion may explore the usefulness of other previously reported parsimonious models (SAILS and HARP-2 clinical trials; Sinha P et al, under submission) as well as the use of varying probability cutpoints for phenotype assignment in comparison to full latent class analysis.

Data from the COVID-19 cohort may be analysed and reported separately.

11.3 Missing Data

Every effort will be made to minimise missing baseline and outcome data in this study. The level and pattern of the missing data in the baseline variables and outcomes will be established by forming appropriate tables and the likely causes of any missing data will be investigated. This information will be used to determine whether the level and type of missing data has the potential to introduce bias into the analysis results for the proposed statistical methods, or substantially reduce the precision of estimates related to treatment effects. If necessary, these issues will be dealt with using multiple imputation or Bayesian methods for missing data as appropriate.

12 DATA MONITORING

12.1 Data Access

Prior to commencement of the study, the PI will give permission for study related monitoring, audits, ethics committee review and inspections, by providing direct access to source data and study related documentation. The patients' confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

12.2 Monitoring Arrangements

The CTU will be responsible for study monitoring. The frequency and type of monitoring will be detailed in the Monitoring Plan and agreed by the study Sponsor. Remote and central monitoring activities will be conducted in accordance with the study Monitoring Plan and will comply with the principles of GCP.

The PI or designee should ensure that access to all study related documents including source documents (to confirm their consistency with CRF entries) are available during any on-site monitoring visits which may take place.

13. TRIAL COMMITTEES

13.1 Trial Management Arrangements

The CI will have overall responsibility for the conduct of the study. The CTU will undertake study management including all clinical study applications (Ethics and Research Governance), site initiation/training, monitoring, analysis and reporting. The Study Co-ordinator will be responsible on a day to day basis for overseeing and co-ordinating the work of the multi-disciplinary study team, and will be the main contact between the study team and other parties involved. Before the study starts, site training will take place to ensure that all relevant essential documents and study supplies are in place and that site staff are fully aware of the study protocol and procedures. The CTU will assist and facilitate in the setting up and co-ordination of the study committees including the Trial Management Group (TMG) and Study Steering Committee (SSC).

13.2 Trial Management Group (TMG)

A Trial Management Group (TMG) will be established and Chaired by the CI. The TMG will have representation on it from the CTU and other investigators/collaborators who are involved in the study and provide study specific expertise. This group will have responsibility for the operational management of the study, and regular meetings of the TMG will be held to discuss and monitor progress. The discussions of the TMG will be formally minuted and a record kept in the TMF.

A TMG Charter will be drawn up to detail the terms of reference of the TMG including roles and responsibilities.

13.3 Study Steering Committee (SSC)

The conduct of the study will be overseen by the SSC. The SSC will take responsibility for monitoring and guiding overall progress, scientific standards, operational delivery and protecting the rights and safety of study patients for the Innovate-UK funded programme of work.

The SSC will include an independent Chair, an independent clinician/trialist, a patient representative, a statistician and the CI. Representatives of the Sponsor/Funder and CTU may attend SSC meetings as observers at the discretion of the Chair. The SSC Charter will document the membership of the committee and outline the terms of reference of the SSC including roles/responsibilities, organisation of meetings, reporting, decision making and the relationship with the other study committees. An inaugural meeting will be held prior to recruitment commencing. Subsequent meetings will be approximately at 6 monthly intervals.

14. REGULATIONS, ETHICS AND GOVERNANCE

The study will comply with the principles of GCP, the requirements and standards set out by the applicable regulatory requirements in the UK and ROI and the UK policy framework for health and social care research.

14.1 Sponsorship

Queen's University Belfast (QUB) will act as Sponsor for the study and the CI will take overall responsibility for the conduct of the study. Separate agreements will be put in place between the Sponsor and each organisation who will undertake Sponsor delegated duties in relation to the management of the study.

14.2 Funding

This study is funded by Innovate UK (Project number: 104639).

14.3 Indemnity

QUB as Sponsor will provide indemnity for the management and design of the study. QUB will provide indemnity for negligent and non-negligent harms caused to patients by the design of the research protocol. The NHS/HSE indemnity scheme will apply with respect to clinical conduct and clinical negligence.

14.4 Competing Interests

The CI and members of the TMG have no financial or non-financial competing interests. The study is funded by Innovate UK. The devices and assays will be provided free of charge by the manufacturer, Randox Laboratories Ltd. Randox Laboratories Ltd had no role in the study design and protocol development and will have no role in the study conduct, data analysis, or data interpretation.

14.5 Ethical Approvals

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. The protocol will be approved by a relevant Research Ethics Committees in the UK and ROI.

14.6 Good Clinical Practice

The study will be carried out in accordance with the principles of the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines (www.ich.org). All members of the study team will be required to have completed GCP training.

14.7 Protocol Compliance

A protocol deviation is defined as an incident which deviates from the normal expectation of a particular part of the study process. Any deviations from the protocol will be fully documented on the protocol deviation form in the CRF.

A serious breach is defined as a deviation from the study protocol or GCP which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the subjects of the study; or
(b) the scientific value of the study

The PI or designee is responsible for ensuring that serious breaches are reported directly to the CTU within one working day of becoming aware of the breach.

Protocol compliance will be monitored by the CTU to ensure that the study protocol is adhered to and that necessary paperwork (e.g. CRFs, patient agreement/consent) is being completed appropriately.

14.8 Protocol Amendments

The investigators will conduct the study in compliance with the protocol given approval/favourable opinion by the Ethics Committee. Changes to the protocol may require ethics committee approval/favourable opinion prior to implementation. The CTU in collaboration with the Sponsor will submit all protocol modifications to the research ethics committees for review in accordance with the governing regulations.

14.9 Patient Confidentiality

In order to maintain confidentiality, all study reports and communication regarding the study will identify the patients by the assigned unique study identifier only. The only exception to this may occur to facilitate the NICTU determining participant mortality by contacting the participant's GP. Computers where information will be stored will be password protected. Patient confidentiality will be maintained at every stage and will not be made publicly available to the extent permitted by the applicable laws and regulations.

14.10 Record Retention

The PI will be provided with an ISF by the CTU and will maintain all study records according to GCP and the applicable regulatory requirements. The TMF will be held by the CTU. On completion of the study, the TMF and study data will be archived by the CTU according to the applicable regulatory requirements and as required by the Sponsor. Following confirmation from the Sponsor the CTU will notify the PI when they are no longer required to maintain the files. If the PI withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept responsibility and this must be documented in writing to the CTU and Sponsor.

15. DISSEMINATION/PUBLICATIONS

15.1 Trial Publications

In the spirit of open research into COVID-19 and in keeping with best practice, data on the COVID-19 cohort will be reported separately, potentially using an earlier time point of 28-day mortality, before the main study completion.

The final statistical report will be provided by the Study Statistician and will inform the final clinical study report; it is anticipated that the study findings will be published in national and international peer reviewed journals and that the preparation of the report will be led by the CI. This will secure a searchable compendium of these publications and make the results readily accessible to the public and health care professionals. In addition, study findings may be presented at both national and international meetings and also to appropriate patient groups.

15.2 Authorship Policy

Authorship will be determined according to the internationally agreed criteria for authorship www.icmje.org.

15.3 Trial Registration

The study will be registered with clinicaltrials.gov.

15.4 Data Sharing Statement

Requests for data sharing will be reviewed on an individual basis by the CI and CTU.

15.5 Data Access

Following the publication of the primary and secondary outcomes there may be scope to conduct additional analyses on the data collected. In such instances formal requests for data will need to be made in writing to the CI via the CTU, who will discuss this with the Sponsor. In the event of publications arising from such analyses, those responsible will need to provide the CI with a copy of any intended manuscript for approval prior to submission.

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