

Study Title: PRognostication of Oxygen Requirment In non-severe SARS-CoV-2 infEction

Short title: PRIORITISE

Clinicaltrials.gov registration: NCT04441372

MSF ERB Ref: 2060

OxTREC Ref: 47-20

Date and Version No: 29 September 2020 version 4.0

Principal Investigators: **Dr. Sakib Burza** (sakib.burza@barcelona.msf.org)

Medical Advisor & Physician

Médecins Sans Frontières Operational Centre Barcelona Athens

Dr. Arjun Chandna (arjun@tropmedres.ac)

Research Physician & Infectious Diseases / Microbiology Registrar
Cambodia Oxford Medical Research Unit

Investigators: **Prof. Nicholas Day, Prof. Arjen Dondorp, Prof. Yoel Lubell, Prof. Paul Turner, Dr. Sophie Yacoub** (University of Oxford)

Endeshaw M Aderie, Dr. Carolina Jimenez, Dr. Mikhael Yosia
(*Médecins Sans Frontières* Operational Centre Barcelona Athens)

Sponsors: University of Oxford and MSF-OCBA

Funders: UK Wellcome Trust

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the authorised individuals from the University of Oxford, *Médecins Sans Frontières* Operational Centre Barcelona Athens (MSF-OCBA), the Investigator Team, and members of the Oxford Tropical Research Ethics Committee (OxTREC), MSF Ethics Review Board (MSF ERB) and national ethics committees of the participating countries, unless authorised to do so. The investigators declare there are no conflicts of interest.

Dr. Sakib Burza

(Principal Investigator)



(Signature)

29 September 2020

(Date)

Dr. Arjun Chandna

(Principal Investigator)



(Signature)

29 September 2020

(Date)

TABLE OF CONTENTS

1. SYNOPSIS.....	4
2. ABBREVIATIONS	4
3. BACKGROUND AND RATIONALE	6
4. OBJECTIVES AND OUTCOME MEASURES.....	11
5. STUDY DESIGN	11
6. PARTICIPANT IDENTIFICATION AND RECRUITMENT	13
6.1. Study Participants	13
6.2. Inclusion Criteria	13
6.3. Exclusion Criteria.....	13
7. STUDY PROCEDURES.....	14
7.1. Recruitment	14
7.2. Informed Consent	14
7.3. Baseline Assessments	15
7.4. Subsequent Visits	15
7.5. Sample Handling	16
7.6. Discontinuation/Withdrawal of Participants from Study	16
7.7. Definition of End of Study	16
8. SAFETY REPORTING.....	17
8.1. Anticipated risks of study-related procedures.....	17
8.2. Infection prevention and control precautions related to COVID-19	17
9. STATISTICS AND ANALYSIS	18
9.1. Description of Statistical Methods.....	18
9.2. The Number of Participants.....	19
9.2. Analysis of Outcome Measures.....	20
10. DATA MANAGEMENT	20
10.1. Access to Data	20
10.2. Data Handling and Record Keeping	20
11. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES.....	21
12. ETHICAL AND REGULATORY CONSIDERATIONS.....	21
12.1. Declaration of Helsinki	21
12.2. Guidelines for Good Clinical Practice	21
12.3. Approvals	22
12.4. Participant Confidentiality	22
12.5. Compensation	22
12.6. Benefits	22

12.7. Reporting.....	22
12.8. Other Ethical Considerations	22
13. FINANCE AND INSURANCE.....	23
13.1. Funding.....	23
13.2. Insurance.....	23
14. PUBLICATION POLICY	23
15. REFERENCES.....	24
16. APPENDIX A: STUDY SITES.....	26
17. APPENDIX B: STUDY FLOW CHART.....	36
18. APPENDIX C: SCHEDULE OF STUDY PROCEDURES	37
19. APPENDIX D: AMENDMENT HISTORY	38

1. SYNOPSIS

Study Title	PR ognostIcation of O xygen R equiremen T In non-severe SARS-CoV-2 infEction (PRIORITISE)	
Study Design	Prospective longitudinal observational study	
Study Participants	Adults with non-severe respiratory illness presenting to healthcare facilities that assess and manage patients with COVID-19	
Planned Sample Size	The sample size will be adaptive, informed by the prevalence of confirmed COVID-19 cases and proportion of participants meeting the primary endpoint (development of a supplemental oxygen requirement). The initial planned sample size is 600 participants per country.	
Planned Study Period	8 months (4-6 months of participant recruitment, depending on evolution of the outbreak)	
	Objectives	Outcome Measures
Primary	To identify clinical and biochemical prognostic markers in adults with laboratory confirmed SARS-CoV-2 infection who do not require oxygen supplementation, with a focus on: <ul style="list-style-type: none"> • aiding safe discharge from a healthcare facility (i.e. a high NPV); • near-term impact on COVID-19 interventions in resource-limited settings (i.e. simple clinico-demographic variables and biochemical markers for which near-patient / POCTs are commercially available or in late-stage development) 	Ability of the markers to predict progression to subsequent need for supplemental oxygen – sensitivity, specificity, NPV, PPV and discrimination (c-index) and calibration (plots of observed probabilities against predicted probabilities) of a prognostic model combining up to four markers
Secondary	To evaluate the field-based performance of a near-patient lateral flow assay for suPAR in adults with non-severe SARS-CoV-2 infection	Correlation of the near-patient lateral flow assay and the instrumented multi-analyte immunoassay gold standard.

2. ABBREVIATIONS

CMC	Christian Medical College, Vellore
COVID-19	Coronavirus Disease-2019
CTSG	Clinical Trials Support Group
CRF	Case report form
CRP	C-reactive protein
eCRF	Electronic Case Record Form
EDTA	Ethylenediaminetetraacetic acid

EPV	Events per variable
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
Icddr,b	International Centre for Diarrhoeal Disease Research, Bangladesh
ICF	Informed consent form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IL-6	Interleukin-6
ISARIC	International Severe Acute Respiratory and Emerging Infection Consortium
LMIC	Low- and middle-income country
LTFU	Loss to follow-up
MICE	Multiple imputation by chained equations
MORU	Mahidol Oxford Tropical Medicine Research Unit
MSF ERB	<i>Médecins Sans Frontières</i> Ethics Review Board
MSF-OCBA	<i>Médecins Sans Frontières</i> Operational Centre Barcelona Athens
NLR	Neutrophil to lymphocyte ratio
NPV	Negative predictive value
ODK	Open Data Kit
OxTREC	Oxford Tropical Research Ethics Committee
PCT	Procalcitonin
PI	Principal investigator
PIS	Participant information sheet
POCT	Point-of-care test
PPE	Personal protective equipment
PPV	Positive predictive value
RDT	Rapid diagnostic test
RT-PCR	Reverse transcription polymerase chain reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome-Coronavirus-2
SEACTN	South and Southeast Asian Community-based Trials Network
SIV	Site initiation visit
SMG	Study Management Group
SOP	Standard operating procedure
sTREM-1	Soluble triggering receptor expressed on myeloid cells-1
suPAR	Soluble urokinase-type plasminogen activator receptor
WHO	World Health Organization

3. BACKGROUND AND RATIONALE

At the time of writing the novel betacoronavirus SARS-CoV-2 has infected nearly 25 million individuals and killed more than 800,000.¹ The most recent epidemiological surveillance curve shows that the number of new infections continues to rise, with over 1.8 million new cases reported in the last week.

Whilst the absolute number of deaths attributable to COVID-19 is substantial, effectively managing high patient volumes is also a major challenge facing health systems, particularly in under-resourced settings.² With an estimated case-fatality rate amongst symptomatic patients of between 1-2%,³ it is vitally important that health workers are able to accurately identify the majority of patients at low risk for progression to severe disease. These patients can be safely discharged away from the health facility ensuring the available resources are allocated to patients most likely to benefit. If low risk patients cannot be readily identified, there is a real risk that health facilities in these regions will be overrun, with consequent substantial avoidable mortality.

Having the ability to prognosticate the need for supplemental oxygen (the main treatment available in these settings) through measurement of parameters available at the time of arrival at a healthcare facility would strengthen the capability to identify those patients presenting with moderate symptoms that can be safely discharged away from the facility. Based on recent estimates, only 20% of all symptomatic patients with COVID-19 develop a requirement for supplemental oxygen.^{4,5}

Although existing prognostic scores have yielded disappointing results in patients with SARS-CoV-2 infection,^{6,7} a number of demographic, clinical and laboratory parameters are associated with a more severe disease course and worse patient outcomes.⁸⁻¹¹ However, to our knowledge few studies have examined the performance of prognostic markers in patients who do not require supplemental oxygen at presentation.¹²⁻¹⁵ Only one of these studies included outpatient or ambulatory care settings and none were conducted in resource-limited settings.¹² Hence, whether measurement of these parameters can inform the decision to safely discharge a patient away from a health facility is as yet unclear.

Main research question

In adults presenting to care with non-severe COVID-19, can subsequent need for supplemental oxygen be predicted from parameters measured at the time of arrival at a healthcare facility?

For the purposes of this study non-severe COVID-19 is defined **as patients with reverse transcription polymerase chain reaction (RT-PCR) confirmed SARS-CoV-2 and presenting with systemic symptoms but not requiring supplemental oxygen therapy.**

We have defined 'yellow flag' systemic symptoms to reflect patients in whom there is a degree of clinical uncertainty as to whether they are safe to send home or should be admitted

for observation. The systemic symptoms are felt to be acceptable surrogates for Grade 3 or 4 on the World Health Organization (WHO) COVID-19 clinical progression scale, i.e. “symptomatic: assistance needed” (Grade 3) or “hospitalised (not for public health isolation reasons): no oxygen therapy” (Grade 4). The advantage of using clearly defined systemic symptoms is that they can be relatively objectively assessed and will provide some standardisation across different settings, where the threshold for hospitalisation or for a patient to report the need for assistance with daily activities may vary.¹⁶

Need for supplemental oxygen (WHO Grade ≥ 5) is defined as a peripheral oxygen saturation $\leq 93\%$ and/or a respiratory rate > 30 breaths per minute and/or a clinical indication to give supplemental oxygen.

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalised: moderate disease	Hospitalised; no oxygen therapy*	4
	Hospitalised; oxygen by mask or nasal prongs	5
Hospitalised: severe diseases	Hospitalised; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, $pO_2/FiO_2 \geq 150$ or $SpO_2/FiO_2 \geq 200$	7
	Mechanical ventilation $pO_2/FiO_2 < 150$ ($SpO_2/FiO_2 < 200$) or vasopressors	8
	Mechanical ventilation $pO_2/FiO_2 < 150$ and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

Figure 1. WHO COVID-19 Clinical Progression Scale.¹⁶ ECMO = extracorporeal membrane oxygenation; FiO_2 = fraction of inspired oxygen; NIV = non-invasive ventilation; pO_2 = partial pressure of oxygen; SpO_2 = oxygen saturation. *if hospitalised for isolation only, record status as for ambulatory patient.

This study will evaluate several clinical and biochemical biomarkers that have been identified as possible predictors of deterioration in patients with COVID-19. The primary objective is to develop a prognostic tool combining up to four markers (including a maximum of two biochemical markers) with a high negative predictive value (NPV) for subsequent supplemental oxygen requirement (WHO Grade ≥ 5). Clinical biomarkers will be limited to simple clinico-demographic variables (for example, age, sex and duration of symptoms) in order to ensure the tool remains as simple as possible. Biochemical biomarkers for which point-of-care (POC) and/or near-patient tests are either already commercially available or in late-stage development have been prioritised (**Table 1**), in order to maximise the chance of translation on to the field within a time period that is useful for the current global pandemic response.

Requirement for supplemental oxygen has been selected as the primary outcome: it is a relatively objective endpoint (based either on measurement of peripheral oxygen saturation [$\text{sO}_2 \leq 93\%$] or a respiratory rate > 30 breaths per minute or a clinical indication to give supplemental oxygen) and from a practical perspective is the main evidence-based therapeutic intervention available in such settings, but in very limited supply in the majority of low- and middle-income countries (LMICs).

Secondary research question

We have identified a near-patient test for a priority biochemical biomarker that has not yet been field-evaluated in LMICs (suPAR). We will evaluate the performance of this test under field conditions. We have selected this test for evaluation because unlike some of the other markers listed in **Table 1** for which near-patient tests also exist, there is a relative paucity of field experience in tropical settings. As all biochemical markers (including suPAR) will be measured using a validated multi-analyte quantitative immunoassay (using the Ella platform), this study will provide an opportunity to evaluate the field performance of this test. If suPAR is found to be a useful prognosticator for patients with non-severe SARS-CoV-2 infection, this may facilitate more rapid translation of the results of this study in to practice.

Study Sites

The study has been designed to be modular and will be implemented at healthcare facilities in LMICs that assess and manage patients with COVID-19. Each participating country will recruit a minimum of 600 participants (subject to the adaptive sample size requirements, see Section 9.2) to power the analysis independently for each country. This ‘over-arching protocol’ describes the study processes that will apply at all sites. Additional site-specific details are provided in the **Appendix A**.

Assumptions, limitations and generalisability

The main assumption that underlies this work is that a validated, rapid, low-cost antigen-based test for SARS-CoV-2 will become available in the near future. There is reason to believe that this will be the case.¹⁷⁻¹⁹ Availability of such a test would allow the prognostic tool developed in this study to be applied to the appropriate patient population at the point of arrival at a health facility. Whilst absence of such a test would undoubtedly limit the utility of the tool, it is likely that when local incidence of patients presenting with confirmed SARS-CoV-2 infection exceeds a threshold, use of the tool could be justified based upon a clinical case definition alone.

An additional limitation of this study is that we will not be able to perform an external geographic validation of the prognostic model, as to do so would require an estimated 1,200 participants to be recruited in one country (to provide a sufficient number of outcome events). To mitigate this risk we will maintain the option that if one particular study site is recruiting well, we will aim to extend recruitment at that site to enrol the necessary number of

participants to perform an external geographic validation. We will inform and seek approval from all relevant regulatory and ethical bodies (international and local) prior to extension of recruitment at any participating study site.

Biomarker	Evidence in COVID-19	Stage of development for near-patient test
Neutrophil:Lymphocyte ratio (NLR)	Multiple studies have identified elevated neutrophil counts and/or decreased lymphocyte counts as poor prognostic indicators in patients with COVID-19. ^{4,9,20}	Quantitative near-patient tests such as the HemoCue WBC DIFF can measure differential leukocyte counts using 10µL of whole blood, with a turnaround time of 5 minutes.
C-reactive protein (CRP)	CRP has been found to be a poor prognostic indicator in hospitalised patients with COVID-19 in China and Germany. ^{9,10,21} Elevated CRP is being used to enrich study populations in trials of novel therapeutics (e.g. TACTIC trial: ISRCTN 11188345) and to inform access to newly licensed treatments (e.g. Remdesivir, UK MHRA).	Numerous commercial POCTs exist for quantitative measurement of CRP, for example the Nycocard CRP test manufactured by Alere, which has a 3 minute turnaround time and requires 5µL of whole blood. It is widely implemented in many resource-limited settings.
D-dimer	Coagulopathy, including multi-system arterial and venous thromboses, is emerging as an important component of COVID-19 pathophysiology. Several studies have established D-dimer as a predictor of poor outcomes in hospitalised patients with COVID-19 and measurement of D-dimer is recommended by the International Society of Thrombosis and Haemostasis in the triage of COVID-19 patients. ^{4,22-24}	Several commercial D-dimer POCTs are available. The RAMP D-dimer test is a quantitative lateral flow assay with a turnaround time of 15 minutes that can be run on whole blood. The DIAcheck C2 machine has also been validated for use in LMIC contexts.
Procalcitonin (PCT)	A number of studies including a recent meta-analysis have indicated that patients with elevated PCT levels at admission are more likely to have severe COVID-19 infection and require ICU admission, although questions remain as to whether raised PCT reflects the severity of SARS-CoV-2 infection or secondary bacterial infections. ²⁵⁻²⁷	There are a number of near-patient tests available for measurement of PCT that are compatible with whole blood samples and can deliver a quantitative result within 15 minutes.
Soluble urokinase-type plasminogen activator receptor (suPAR)	suPAR has demonstrated prognostic utility in adults with COVID-19 in the UK, Denmark and Greece. It has been shown to identify patients at high risk of respiratory failure and those safe for discharge from hospital. ^{11,28,29}	The suPARnostic® Quick Triage Test which is commercially available from Virogates measures suPAR quantitatively. The test needs 10µL of plasma. Each assay delivers a quantitative result in 25 minutes and costs USD 20.
Interleukin-6 (IL-6)	Several studies, including a recent meta-analysis, have demonstrated that elevated IL-6 levels predict the risk of severe COVID-19 infection, ARDS and respiratory failure. ^{4,10,27,30} In addition, IL-6 was identified as the best predictor of oxygen requirement in a cohort of Swiss patients (van Singer, in submission). Many clinical trials are investigating the utility of anti-IL-6 therapy in patients with COVID-19.	Quantitative IL-6 POCTs are available, for example the Milenia QuickLine IL-6 rapid lateral flow assay. Each test costs 20 USD, requires 100µL of plasma or serum and has a 20 minute turnaround time. The sensitivity of the Milenia IL-6 test is 50pg/mL, however some studies suggest that the threshold for predicting severe COVID-19 might be lower (e.g. 35pg/mL). Prototypes for other rapid assays are currently being evaluated. ³¹
Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1)	sTREM-1 has been shown to predict respiratory failure in hospitalised patients with COVID-19 (van Singer, in submission).	Prototype quantitative lateral flow assays for sTREM-1 are being developed (FIND, personal communication).

Table 1. Biochemical biomarkers for batched retrospective measurement in the study, and stage of readiness for point-of-care or near-patient tests. **Green = test in routine use in LMIC settings; Orange = test available but not in routine use in LMIC settings; Red = prototype test available.** A number of other biochemical markers (IL-1Ra, IL-8, IL-10, CXCL-10/IP-10 and Ang-2)³²⁻³⁴ have been identified as poor prognostic indicators in patients with COVID-19. Whilst near-patient tests do not yet exist for these markers, a number of them have shown promise as early markers of severity in patients with SARS-CoV-2 infections and we will include these on the multi-analyte panel used in this study.

4. OBJECTIVES AND OUTCOME MEASURES

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure
Primary		
To identify clinical and biochemical prognostic markers in adults with laboratory confirmed SARS-CoV-2 infection who do not require oxygen supplementation, with a focus on: <ul style="list-style-type: none"> • aiding safe discharge from a healthcare facility (i.e. a high NPV); • near-term impact on COVID-19 interventions in resource-limited settings (i.e. simple clinico-demographic variables and biochemical markers for which near-patient / POCTs are commercially available or in late-stage development) 	Ability of the markers to predict progression to subsequent need for supplemental oxygen – sensitivity, specificity, NPV, PPV and discrimination (c-index) and calibration (plots of observed probabilities against predicted probabilities) of a prognostic model combining up to four markers	Clinical and biochemical biomarkers at enrolment (D0), need for supplemental oxygen by D14
Secondary		
To evaluate the field-based performance of a near-patient lateral flow assay for suPAR in adults with non-severe SARS-CoV-2 infection.	Correlation of the near-patient lateral flow assay and the instrumented multi-analyte immunoassay gold standard.	suPAR measured using a near-patient lateral flow assay and the instrumented multi-analyte immunoassay at enrolment (D0)

5. STUDY DESIGN

This is a prospective longitudinal observational study. Adult patients (aged ≥ 18 years) meeting a clinical case definition of non-severe symptomatic COVID-19, who give informed consent, will be recruited.¹⁶

Routine demographic variables will be extracted from the participant's clinical record and recorded on electronic Case Record Forms (eCRFs). A venous blood sample (10 ml) will be collected for measurement of biochemical biomarkers (**Table 1**).

Upper respiratory tract specimens (nasopharyngeal and oropharyngeal swabs) will be collected for RT-PCR of SARS-CoV-2 unless a respiratory specimen has already been collected for RT-PCR as per routine clinical care. The expectation is that the existing system being utilised by the government will be used, however in case of disruption or lack of availability, we will ensure that adequate sample collection, storage and alternative testing facilities are available. If validated antigen-based rapid diagnostic test (RDT) platforms become available during the study and are endorsed by the government, these will be utilised to guide recruitment.

Participants will be provided with routine care, which will not be affected in any way by participation in the study. Study samples collected for measurement of biochemical biomarkers will be batched and run retrospectively. Results will not be available to inform patient management.

Participants will be followed-up telephonically at day 7 (window period: +2 days) and day 14 (window period: +4 days) from date of enrolment to determine outcome, with the primary endpoint being development of an oxygen requirement (reported and/or documented peripheral oxygen saturation $\leq 93\%$ or respiratory rate > 30 breaths per minute or clinical indication to give supplemental oxygen). Participants who are admitted at the study site will be followed-up daily during admission to determine whether they progress to meet the primary endpoint.

Participants who are confirmed not to be infected with SARS-CoV-2 (a negative RT-PCR result) after enrolment will be telephoned to inform them of their result and removed from the study. To be explicit, if the participant re-presents to the sites with new or persistent symptoms consistent with COVID-19 within the same illness episode, they will be screened and if eligible, will be offered to enroll again into the study (including re-testing RT-PCR); however we expect this to be very few cases. All participants will remain in the study until confirmation of a negative RT-PCR result, successful completion of D14 follow-up or being declared lost-to-follow-up (see below).

If telephone contact attempts are unsuccessful for the D14 follow-up, a home visit will be attempted and/or relevant information will be extracted from their medical record if they have sought care since enrolment (see Section 7.4). For practical reasons, outreach follow-up will not be attempted if the participant is uncontactable at the D7 follow-up. For details on infection prevention and control precautions that will be implemented, see section 8.2. If the outreach visit for the D14 follow-up is unsuccessful participants will be declared lost-to-follow-up (LTFU) and further follow-up attempts will not be made. However, if it is established that the reason for inability to contact the participant is that they have been admitted to a health care facility, they will not be declared LTFU until the study team are able to establish contact and determine if the participant has met the primary endpoint or not (i.e. received or required oxygen supplementation). If a participant has ongoing concerns about their health at the D7 or D14 follow-up they will be advised to re-attend the treatment site for assessment by the clinical team.

Venous blood samples for biomarkers will be processed and stored at a minimum of -20°C. Biochemical biomarkers will be measured using the Ella platform, an instrumented multi-analyte immunoassay for quantification of biochemical biomarkers.³⁵ In addition, suPAR will also be measured using a quantitative near-patient lateral flow assay. Samples will be batched and processed at intervals throughout the study to ensure results from the study are available as rapidly as possible, and can inform subsequent patient management during the current COVID-19 pandemic response. Given that understanding of SARS-CoV-2 pathophysiology is rapidly advancing and new tools for patient management are continually being developed, leftover sample aliquots will be stored for use in future ethically-approved studies that share similar aims.

6. PARTICIPANT IDENTIFICATION AND RECRUITMENT

6.1. Study Participants

Adults with symptomatic confirmed or suspected non-severe SARS-CoV-2 infection presenting to the study site.

6.2. Inclusion Criteria

The participant may enter the study if ALL of the following apply:

1. Aged ≥ 18 years, and willing and able to give informed consent and comply with study procedures;
2. RT-PCR or antigen test positive for SARS-CoV-2 during current illness¹
3. Systemic manifestation of SARS-CoV-2 infection defined as:
Breathing difficulty
OR
History of fever during current illness **AND** chest pain OR abdominal pain OR loose stool
OR severe myalgia

6.3. Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

1. Requires supplemental oxygen² or mechanical ventilation (invasive / non-invasive) at presentation;
2. Laboratory confirmed SARS-CoV-2 infection (virological or serological) during a previous illness episode.

¹ Patients presenting without virologically-confirmed SARS-CoV-2 but meeting all other eligibility criteria will be recruited. They will be removed from the study if they are subsequently confirmed to be negative for SARS-CoV-2 via RT-PCR (see Section 5).

² SpO₂ \leq 93% OR respiratory rate $>$ 30 breaths per minute OR clinical indication to give supplemental oxygen.

7. STUDY PROCEDURES

7.1. Recruitment

Research staff will be present in the outpatient and triage locations of the study sites. Screening and enrolment will occur after patient has been assessed and initial management plan formulated by the treating health worker. Consecutive adults will be screened and those who meet the eligibility criteria and who provide informed consent will be recruited. The research staff will explain the purpose of the study and confirm the participant meets all the eligibility criteria (sections 6.2 and 6.3) before asking consent to participate. The person seeking consent will not be the potential participant's treating healthcare worker to avoid therapeutic misconception. The clinical record will be reviewed to confirm eligibility. A log of all screened patients will be maintained, to record the eligibility and refusal rates. Recruitment is anticipated to take between four and six months.

7.2. Informed Consent

Research staff will ask patients who meet all eligibility criteria to provide informed consent to participate in the study. Written versions of the Participant Information Sheet (PIS) and Informed Consent Form (ICF) will be presented to the participant detailing no less than: the exact nature of the study; what it will involve for them; the implications and constraints of the protocol; and the risks and benefits. It will be clearly stated that the participant is free to withdraw from the study at any time and for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. Informed consent will also include a clause for use of data and/or samples in other ethically-approved studies with similar aims. Participants can explicitly decide to opt out of this clause.

Participants will be allowed the opportunity to question the Investigator or other independent parties to decide whether they will participate in the study. Written informed consent will then be obtained by means of participant-dated signature (or impartial witness counter-signed thumbprint for illiterate participants) and dated signature of the person who presented and obtained the informed consent. Participants must personally sign/thumbprint and date the latest approved version of the ICF before any study specific procedures are performed (specifically the blood draw in this study). The person who obtained the consent will be suitably qualified and experienced, and have been authorised to do so by the site Principal Investigator (PI). One copy of the signed ICF will be given to the participant. One copy will be retained at the study site.

7.3. Baseline Assessments

Baseline information (demographics, comorbidities, recent medications, duration of illness, presenting clinical syndrome and vital signs) will be extracted from the participant's clinical record. Any data that is unclear or not contained in the clinical record will be obtained by brief interview with the participant following the informed consent process. Research staff will draw a venous blood sample (10ml) and collect nasopharyngeal and oropharyngeal swabs, if these have not already been collected through routine screening processes.

7.4. Subsequent Visits

At **7 days** (window period: +2 days). Follow-up will be via telephone to ascertain vital status, resolution/persistence of symptoms and any treatment sought/received since enrolment, in particular whether the participant has received supplemental oxygen therapy (via any modality e.g. nasal prongs, face mask, etc.) since enrolment, and their peripheral oxygen saturations and/or respiratory rate prior to commencement of therapy. Information will be cross-checked on the participant's medical records if required (for example, if they have sought care or been admitted to hospital since enrolment and D7). For participants in whom it is anticipated telephone follow-up will be difficult (for example, lack of mobile phone ownership), arrangements will be made for face-to-face follow-up, either via return to the study site or outreach follow-up by the research staff. If a participant reports worsening symptoms on D7, they will be advised to return to the study site for assessment by the clinical team.

At **14 days** (window period: +4 days). Follow-up will be via telephone to ascertain vital status, resolution/persistence of symptoms and any treatment sought/received since enrolment, in particular whether the participant has received supplemental oxygen therapy (via any modality e.g. nasal prongs, face mask, etc.) since enrolment, and their peripheral oxygen saturations and/or respiratory rate prior to commencement of therapy. Information will be cross-checked on the participant's medical records if required (for example, if they have sought care or been admitted to hospital between D7 and D14). For participants in whom it is anticipated telephone follow-up will be difficult (for example, lack of mobile phone ownership), arrangements will be made for face-to-face follow-up, either via return to the study site or outreach follow-up by the research staff. For participants who are uncontactable on D14 a home visit will be attempted to collect the necessary information. If a participant reports persistent or worsening symptoms on D14, they will be advised to return to the study site for assessment by the clinical team.

For participants who are admitted to the study site, daily follow-up will be conducted to determine if they have met the primary endpoint (development of a supplemental oxygen requirement).

Telephone follow-up is expected to be possible at D7 and D14 in the majority of participants. An overview of planned data collection at baseline assessment and subsequent visits is provided in **Appendix C**.

7.5. Sample Handling

Venous blood samples will be collected via venepuncture by research staff. The maximum total blood volume collected will be 10 ml. Samples will be collected in ethylenediaminetetraacetic acid (EDTA) tubes and stored at 2-8°C. Full blood count samples will be batched and run retrospectively within 24 hours. Biochemical biomarker samples will be stored for a maximum of four hours prior to centrifugation. Within 24 hours, centrifuged plasma will be stored at -20°C or below without freeze-thaw. Sample aliquots will be thawed overnight at between 2-8°C and aliquoted at room temperature prior to assay performance. Samples will be batched and biochemical biomarkers will be quantitatively measured using an instrumented multi-analyte immunoassay on the Ella platform.³⁵ suPAR will also be measured using a quantitative near-patient lateral flow assay.

Remaining samples will be stored at -80°C for long-term use in future ethically-approved studies with similar aims. The retention of samples for use in future studies will be explained to participants before they are asked to provide consent to participate. They will be able to opt out of this, without affecting their participation in this study. Consenting participants may rescind their consent at a later date and refuse to permit the use of their samples or data at any time in the future, up until the completion of the study (see section 10.2).

In the event that a respiratory swab is not collected for SARS-CoV-2 RT-PCR testing as part of routine clinical care, combined nasopharyngeal and oropharyngeal swabs will be collected from all participants. RT-PCR for SARS-CoV-2 will be performed and results acted upon in accordance with local and national guidelines.

7.6. Discontinuation/Withdrawal of Participants from Study

Each participant has the right to withdraw from the study at any time up until the completion of the study (final follow-up of the final participant).

In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- 1) Ineligibility (arising during the study or retrospectively having been overlooked at screening, for example, a participant who is subsequently found to be out of the target age range);
- 2) Major protocol deviations as defined in the statistical analysis plan.

Under such circumstances the decision will be communicated to the participant and an explanation for their removal from the study provided.

If withdrawal from the study occurs before data collection is complete, and the reason for withdrawal is not a significant protocol violation to render the participant's data invalid, the participant will be included in the final dataset. The reason for withdrawal will be recorded and withdrawn participants will be replaced.

7.7. Definition of End of Study

The end of the study is the date of the last D14 follow-up of the last participant.

8. SAFETY REPORTING

This is an observational study. Participants will be provided with routine care and no alterations to their treatment will occur as a result of the study, whether or not a participant consents to participate.

8.1. Anticipated risks of study-related procedures

Study-related procedures will be the drawing of a venous blood sample (10 ml) on the day of enrolment (D0). Pain will be caused due to venepuncture. In addition, there is a small risk that a minor bruise may be caused due to traumatic venepuncture. These risks will be minimised by ensuring that all research staff are qualified health professionals with experience in venepuncture and that Standard Operating Procedures (SOP) are in place and followed.

Discomfort will be caused by collection of respiratory swabs. Collection of respiratory swabs are part of routine clinical care and not a study-specific procedure. However, as detailed in Sections 5 and 7.5, if the existing testing system becomes disrupted or unavailable the study will ensure that alternative sample collection, storage and testing facilities are available. Research staff will be trained in collection of respiratory specimens and study-specific SOPs will be in place and followed.

A minority of participants will be asked to re-attend the study site for face-to-face follow-up (for example, those who don't own a mobile telephone). It is possible that some participants may require public transport, which may pose an increased risk of SARS-CoV-2 transmission. Participants will be provided with basic infection prevention and control advice (for example, appropriate hand hygiene and use of face masks) to mitigate this increased risk.

All telephone contacts will follow a standard introductory narrative that will include processes to correctly identify the participant, and then confirmation that the time of contact is appropriate (taking into consideration any privacy concerns for the participant). If the time of the contact is inconvenient from the participant's perspective for whatever reason, the researcher will call back at a more convenient time as recommended by the participant.

8.2. Infection prevention and control precautions related to COVID-19

This study will take place within existing COVID-19 treatment facilities, as such standard protective measures are already established and will continue through the duration of this study. Explicitly, the following mitigation measures will minimise the risk of SARS-CoV-2 transmission related to study procedures:

1. Training (for example, Site Initiation Visits [SIVs]) will be conducted online whenever possible;
2. For face-to-face training physical distancing will be implemented whenever practical;
3. Frontline staff are provided with Personal Protective Equipment (PPE), in line with WHO recommendations and local requirements, for all interactions with participants, including biological sample collection;

4. Appropriate biological sample handling and management in accordance with local and international standards;
5. Follow-up on D7 and D14 will be conducted by telephone for the majority of participants. Face-to-face follow-up (via return to study site or community outreach) will only be used for participants who do not own a mobile telephone or those who are uncontactable at D14. In the case of face-to-face home visits, face masks will be provided to the participant and family to minimise risk of transmission;
6. Diagnostic and treatment coverage for all research staff.

9. STATISTICS AND ANALYSIS

9.1. Description of Statistical Methods

Raw data will be processed in accordance with best practices for raw data management to identify any inaccuracies or incompleteness in advance of the statistical analysis. All interval variables will be checked and summarised in terms of maximum and minimum values. Minimum and maximum values will be compared against the nominal maximum and minimum value of each variable, and implausible values will be flagged. All exposure (predictor) and response (outcome) variables will be summarised and reported for the study using descriptive statistics. Interval variables will be summarised and reported in terms of number, mean and median, standard deviation, and interquartile range. Categorical variables will be summarised and reported in terms of frequency distribution.

A full case analysis will be conducted if the overall amount of missing data is less than 5%. If the fraction of missing data is more than 5% then multiple imputation (likely multiple imputation by chained equations [MICE]) will be used to reduce sampling variability from the imputation process and make use of all data. Regression estimates will be combined using Rubin's rule.

It is known that biomarker data might be non-normally distributed. The relationships between predictor and outcome variables will be explored and transformations used if necessary in the case of non-linear relationships. The final set of candidate variables included in the model will be selected on the basis of biological plausibility, after exploring relationships between predictors, for example by using scatterplots to evaluate correlations as well as data completeness (prior to imputation). Stepwise methods will also be used for exploratory analyses to help inform variable selection.

Logistic regression will be used to derive a prediction model with clinico-demographic variables and biochemical biomarkers that discriminate the outcome groups. Bootstrapping will be used to minimise bias and optimism as part of an internal validation. Model performance will be assessed using a c-index (with 95% confidence intervals) to assess discrimination, as well as creating plots of average observed probability against predicted probability to assess calibration.

Analysis and derivation of the model will be performed independently for each participating country. A Statistical Analysis Plan will be written by the study statistician. If sufficient participants are recruited to permit external validation of the model (as detailed in Section 3) an addendum to the Statistical Analysis Plan will be written to explain how this will be conducted.

9.2. The Number of Participants

A pragmatic approach to sample size estimation has been adopted, considering the feasibility of conducting operational research in the context of an evolving pandemic. We anticipate that it will be feasible to recruit up to five participants per day, which should allow data collection to be completed within a four-month period so that results from this study have a reasonable chance of being implemented within the active life of the current pandemic.

Recruitment will be consecutive (up to the maximum number of participants that is feasible to recruit each day) until the target sample size is achieved. We have followed recent guidance to estimate the required sample size to build the prediction model,^{36,37} and allowed for the following assumptions:

- 5% LTFU; and
- evaluation of up to four candidate predictors;

We anticipate that a sample size of 600 participants (i.e. 570 participants after allowing for LTFU) will power the study for the following scenarios (displayed in **Table 2**), based on varying R² Nagelkerke estimates.

R ² Nagelkerke	PREVALENCE: Sample size (events)								
	5%	6%	7%	8%	9%	10%	11%	12%	13%
0.15	EPV=8.9	EPV=9.6	EPV=10.2	EPV=10.8	EPV=11.4	EPV=12.0	EPV=12.6	EPV=13.2	EPV=13.8
	712(35.6)	638(38.3)	583(40.8)	541(43.3)	508(45.8)	482(48.2)	460(50.6)	441(53)	426(55.3)
0.16	EPV=8.3	EPV=8.9	EPV=9.5	EPV=10.1	EPV=10.7	EPV=11.3	EPV=11.8	EPV=12.4	EPV=12.9
	666(33.3)	596(35.8)	545(38.2)	506(40.5)	475(42.8)	450(45)	430(47.3)	412(49.5)	398(51.7)
0.17	EPV=7.8	EPV=8.4	EPV=9.0	EPV=9.5	EPV=10.0	EPV=10.6	EPV=11.1	EPV=11.6	EPV=12.1
	626(31.3)	560(33.6)	512(35.8)	475(38)	446(40.2)	423(42.3)	403(44.4)	387(46.4)	373(48.5)
0.18	EPV=7.4	EPV=7.9	EPV=8.4	EPV=9.0	EPV=9.5	EPV=10.0	EPV=10.4	EPV=10.9	EPV=11.4
	590(29.5)	528(31.7)	482(33.8)	448(35.8)	420(37.8)	398(39.8)	380(41.8)	364(43.7)	351(45.7)
0.19	EPV=7.0	EPV=7.5	EPV=8.0	EPV=8.5	EPV=8.9	EPV=9.4	EPV=9.9	EPV=10.3	EPV=10.8
	558(27.9)	499(29.9)	456(31.9)	423(33.8)	397(35.7)	376(37.6)	359(39.4)	344(41.3)	332(43.1)
0.2	EPV=6.6	EPV=7.1	EPV=7.6	EPV=8.0	EPV=8.5	EPV=8.9	EPV=9.3	EPV=9.8	EPV=10.2
	529(26.5)	473(28.4)	432(30.2)	401(32.1)	376(33.9)	356(35.6)	340(37.4)	326(39.1)	314(40.8)

Table 3. Sample size scenarios indicating number of participants / outcome events required for different values of R² to derive a prediction model from four candidate predictors. Yellow squares indicate scenarios that a sample size of 570 participants would be adequate for. EPV = events per variable.

We believe that an estimate of 8% for the proportion of participants who do not have a supplemental oxygen requirement at presentation but will develop one over the next 14 days (meet the primary endpoint) is reasonable.¹²⁻¹⁵ The Study Management Group (SMG; section 10.2) will monitor recruitment, including the proportion of participants who meet the primary

endpoint, and the recruitment strategy and target sample size will be adjusted after the first 100 participants are recruited (i.e. an adaptive sample size – for example, if more than 8% of participants progress to meet the primary endpoint the sample size will be reduced and vice versa).

9.3. Analysis of Outcome Measures

Analysis will be performed using data from all participants, irrespective of whether they were subsequently LTFU or withdrew from the study (unless withdrawal was a result of a major protocol violation). Imputation methods (for example, MICE) will be used to account for missing data points, as appropriate. A sensitivity analysis will be conducted with and without missing data. Logistic regression will be used to identify clinico-demographic variables and/or biochemical biomarkers that discriminate the outcome groups. Performance will be assessed using a c-index (and 95% confidence interval) for discrimination, as well as creating plots of average observed probability against predicted probability for calibration.

10. DATA MANAGEMENT

10.1. Access to Data

Direct access will be granted to authorised representatives from the University of Oxford, MSF-OCBA, and national ethics committees of the participating countries for monitoring and/or audit of the study to ensure compliance with regulations.

10.2. Data Handling and Record Keeping

The SMG will be responsible for the establishment, monitoring and review of data collection procedures. Clinical study data will be recorded on eCRFs and entered on to a GCP-compliant data management system. The database is password-protected and includes internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate. The study data management plan outlines measures that will be carried out to ensure security and quality of the data.

Measures will be taken to ensure non-disclosure of information that is potentially harmful to participants. Paper records (for example, patient identifiable information for the purposes of follow-up, the screening logs and signed ICFs) will be kept in locked cabinets; electronic data will only be accessible to staff with user accounts and passwords. Enrolment logbooks will be destroyed after the completion of the study. After this point no link between patient identifiable details and study data will remain. Electronic data will be preserved indefinitely.

Data stored in the database may be shared according to the terms defined in the MORU and MSF data sharing policies with data repositories or other researchers to use in the future.

All personal information will be pseudonymised, so that no individual can be identified. Access to the archive data will follow a formal process of application to the MORU and/or MSF Data Access Committees.

Biological samples will be collected by the research staff, and processed, stored and archived in secure laboratories. Laboratory freezer management systems will be used to ensure secure storage and archiving of all samples.

11. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in compliance with this protocol, International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP) and any applicable regulatory requirement(s). Monitoring will be performed to ensure compliance to the study protocol and applicable guidelines and regulations. Blood samples will be processed, stored and transported in accordance with local laboratory SOPs.

The process of taking Informed Consent will be Quality Controlled. All research staff will be trained in accordance with GCP, and will have completed training for Ethical Research Practice. Any newly hired research staff will be observed by experienced research staff when they consent potential study participants. The experienced staff will assess performance, provide feedback as necessary and confirm that the new research staff are satisfactorily able to take consent independently.

Data validation will be performed to identify errors or discrepancies and thus ensure completeness, validity and accuracy of data. Data validation activities will include:

- 1) Automated checks that will be built into the study database to identify missing values and to flag inconsistent or invalid data during data entry.
- 2) Post-entry checks that will be performed through profiling of the study data using statistical software.

Monitoring will be performed by the MORU Clinical Trials Support Group (CTSG) according to a risk-based monitoring plan to ensure compliance to the study protocol and applicable guidelines and regulations.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

12.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with GCP.

12.3. Approvals

The protocol, informed consent form and participant information sheet will be submitted to OxTREC, the MSF ERB and national ethics committees of the participating countries for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all amendments to the original approved documents. No amendments will be implemented prior to approval unless there is an immediate and significant risk to study participants.

12.4. Participant Confidentiality

The participants will be identified only by a participant identification number on all study documents and any electronic database, with the exception of the signed ICF and the study recruitment logbook, where participant name, address and telephone details will be recorded, for the purposes of contacting participants for follow-up. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the General Data Protection Regulation (GDPR), which requires that personal data must not be kept as identifiable data for longer than necessary for the purposes concerned.

12.5. Compensation

Participants will not be paid for their participation in the research. For participants who return to the study site for their D7 or D14 follow-up, they will be paid to compensate for loss of daily wage and travel allowance, in accordance with locally-agreed protocols.

12.6. Benefits

Individual participants are not expected to directly benefit from participating in the study. However, the development of prognostic tool for patients presenting with COVID-19 has the potential to benefit future patients who present to health facilities in resource-limited settings during the current pandemic and beyond.

12.7. Reporting

The PIs shall submit an End of Study Report to OxTREC, the MSF ERB and the national ethics committees of the participating countries within the timeframe required by each committee (12 months).

12.8. Other Ethical Considerations

Participants will receive a telephone call from a research team member on D7 and D14. If participants have persistent or worsening symptoms they will be advised to return for review by the clinical team as per standard of care. Hence, the D7 and D14 follow-up will provide an opportunity to signpost options to seek care and individual participants may benefit from this additional 'safety-net'. Processes will be followed as described earlier in the protocol to ensure that participant privacy concerns are addressed during telephone contacts.

Depending on the location of the study sites vulnerable populations may be included in the study. Whenever this is the case these details will be provided in **Appendix A**.

13. FINANCE AND INSURANCE

13.1. Funding

Principal funding for this study is from the UK Wellcome Trust (Wellcome Innovations Flagship Award to the South and Southeast Asian Community-based Trials Network [SEACTN] project: 215604/Z/19/Z).

13.2. Insurance

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

14. PUBLICATION POLICY

All results that are generated from this study will be published in open access mode in accordance with Wellcome Trust and MSF publication policies. All publications will abide by the International Committee of Medical Journal Editors (ICMJE) recommendations for the role of authors and contributors.

The results of the study (positive or negative) will be summarised in lay language and disseminated to key stakeholders and community leaders, with an opportunity for any questions and clarifications ensured. Country health authorities will also be engaged proactively with the results to ensure the opportunity for uptake of the tool is present. Where feasible, participants will also be contacted and the results of the study relayed.

15. REFERENCES

1. WHO. Coronavirus disease (COVID-19) Weekly Epidemiological Update - 30 August 2020, 2020.
2. Cash R, Patel V. Has COVID-19 subverted global health? *Lancet* 2020; **395**(10238): 1687-8.
3. Wu JT, Leung K, Bushman M, et al. Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China. *Nat Med* 2020; **26**(4): 506-10.
4. Wu C, Chen X, Cai Y, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med* 2020.
5. WHO. Oxygen sources and distribution for COVID-19 treatment centres, 2020.
6. Ferreira M, Blin T, Collercandy N, et al. Critically ill SARS-CoV-2-infected patients are not stratified as sepsis by the qSOFA. *Ann Intensive Care* 2020; **10**(1): 43.
7. Aziz S, Arabi YM, Alhazzani W, et al. Managing ICU surge during the COVID-19 crisis: rapid guidelines. *Intensive Care Med* 2020; **46**(7): 1303-25.
8. The OpenSAFELY Collaborative, Williamson E, Walker AJ, et al. OpenSAFELY: factors associated with COVID-19-related hospital death in the linked electronic health records of 17 million adult NHS patients. *medRxiv* 2020.
9. Luo X, Zhou W, Yan X, et al. Prognostic value of C-reactive protein in patients with COVID-19. *Clin Infect Dis* 2020.
10. Herold T, Jurinovic V, Arnreich C, et al. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J Allergy Clin Immunol* 2020; **146**(1): 128-36 e4.
11. Rovina N, Akinosoglou K, Eugen-Olsen J, Hayek S, Reiser J, Giamarellos-Bourboulis EJ. Soluble urokinase plasminogen activator receptor (suPAR) as an early predictor of severe respiratory failure in patients with COVID-19 pneumonia. *Crit Care* 2020; **24**(1): 187.
12. Prieto-Alhambra D, Ballo E, Coma-Redon E, et al. Hospitalization and 30-day fatality in 121,263 COVID-19 outpatient cases. *medRxiv* 2020.
13. Shi J, Li Y, Zhou X, et al. Lactate dehydrogenase and susceptibility to deterioration of mild COVID-19 patients: a multicenter nested case-control study. *BMC Med* 2020; **18**(1): 168.
14. Shi Y, Yu X, Zhao H, Wang H, Zhao R, Sheng J. Host susceptibility to severe COVID-19 and establishment of a host risk score: findings of 487 cases outside Wuhan. *Crit Care* 2020; **24**(1): 108.
15. Cen Y, Chen X, Shen Y, et al. Risk factors for disease progression in patients with mild to moderate coronavirus disease 2019-a multi-centre observational study. *Clin Microbiol Infect* 2020.
16. WHO Working Group on the Clinical Characterisation and Management of Covid-19 Infection. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis* 2020.
17. Collier DA, Assennato SM, Sithole N, et al. Rapid point of care nucleic acid testing for SARS-CoV-2 in hospitalised patients: a clinical trial and implementation study. *medRxiv* 2020.
18. Indian Council of Medical Research. Advisory on Use of Rapid Antigen Detection Test for COVID-19. 2020.
19. Dutta SS. First antigen based COVID-19 testing kit approved in India, expected to ramp up testing capacity manifold. *The New Indian Express*. 2020.
20. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**(10223): 497-506.

21. Wang G, Wu C, Zhang Q, et al. C-Reactive Protein Level May Predict the Risk of COVID-19 Aggravation. *Open Forum Infect Dis* 2020; **7**(5): ofaa153.
22. Zhang L, Yan X, Fan Q, et al. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. *J Thromb Haemost* 2020; **18**(6): 1324-9.
23. Thachil J, Tang N, Gando S, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost* 2020; **18**(5): 1023-6.
24. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**(10229): 1054-62.
25. Lippi G, Plebani M. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. *Clin Chim Acta* 2020; **505**: 190-1.
26. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020.
27. Liu F, Li L, Xu M, et al. Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. *J Clin Virol* 2020; **127**: 104370.
28. Eugen-Olsen J, Altintas I, Tingleff J, et al. Low levels of the prognostic biomarker suPAR are predictive of mild outcome in patients with symptoms of COVID-19 - a prospective cohort study. *medRxiv* 2020.
29. Arnold DT, Attwood M, Barratt S, et al. Blood parameters measured on admission as predictors of outcome for COVID-19; a prospective UK cohort study. *medRxiv* 2020.
30. Aziz M, Fatima R, Assaly R. Elevated interleukin-6 and severe COVID-19: A meta-analysis. *J Med Virol* 2020.
31. Sene IdS, Costa V, Braz DC, et al. A Point of Care Lateral Flow Assay for Rapid and Colorimetric Detection of Interleukin 6 and Perspectives in Bedside Diagnostics. 2020. <https://maplespub.com/article/A-Point-of-Care-Lateral-Flow-Assay-for-Rapid-and-Colorimetric-Detection-of-Interleukin-6-and-Perspectives-in-Bedside-Diagnostics>.
32. Smadja DM, Guerin CL, Chocron R, et al. Angiopoietin-2 as a marker of endothelial activation is a good predictor factor for intensive care unit admission of COVID-19 patients. *Angiogenesis* 2020.
33. Yang Y, Shen C, Li J, et al. Exuberant elevation of IP-10, MCP-3 and IL-1ra during SARS-CoV-2 infection is associated with disease severity and fatal outcome. *medRxiv* 2020.
34. Zhao Y, Qin L, Zhang P, et al. Longitudinal COVID-19 profiling associates IL-1Ra and IL-10 with disease severity and RANTES with mild disease. *JCI Insight* 2020.
35. Leligdowicz A, Conroy AL, Hawkes M, et al. Validation of two multiplex platforms to quantify circulating markers of inflammation and endothelial injury in severe infection. *PLoS One* 2017; **12**(4): e0175130.
36. Riley RD, Ensor J, Snell KIE, et al. Calculating the sample size required for developing a clinical prediction model. *BMJ* 2020; **368**: m441.
37. Riley RD, Snell KI, Ensor J, et al. Minimum sample size for developing a multivariable prediction model: PART II - binary and time-to-event outcomes. *Stat Med* 2019; **38**(7): 1276-96.

16. APPENDIX A: STUDY SITES

1. Cox Bazar, Bangladesh

Partner institute

International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh (icddr,b)

Co-investigators

Dr. Shomik Mohammad (icddr,b, Dhaka, Bangladesh)

Dr. Dinesh Mondal (icddr,b, Dhaka, Bangladesh)

Dr. Ariful Bashar (Infectious Disease Hospital, Dhaka, Bangladesh)

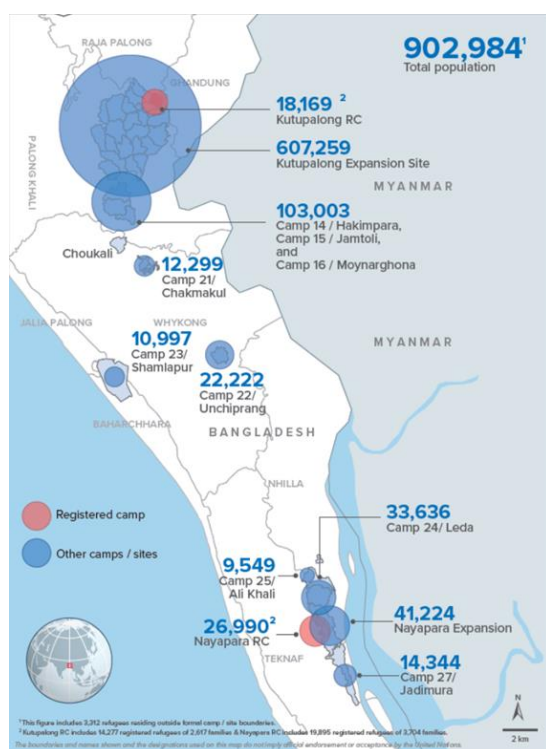
Funding (for site costs)

UK Wellcome Trust and MSF-OCBA

Rationale

COVID-19 has already started to hit extremely vulnerable populations in refugee camps and conflict areas. In Bangladesh, cases within the Rohingya refugee megacamps have already been reported, and local transmission is ongoing. Such contexts already suffer from a lack of medical facilities and even a moderate number of COVID-19 cases will overburden existing, and proposed, capacity.

Site description



The study will be conducted at two MSF-OCBA COVID-19 treatment facilities in Cox Bazar, Bangladesh, both located in the southern Upazilla of Teknaf (Nayapara and Unchiparang/Camp 22 treatment centres). Both have the capacity for screening and admission of patients, and cater to host and refugee populations. Both sites are additional sites that have been created in response to the COVID-19 pandemic, and offer only services related to COVID-19. Both sites are recognised by the government and Health Clusters as COVID-19 management facilities. The study will be conducted in partnership with the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), following and utilising the same study architecture and agreements for the Spot Sepsis study (MSF ERB 1967).

Enrolment procedures

Research staff will be present in the outpatient and triage locations of the study sites. Screening and enrolment will occur after patient has been assessed and initial management plan formulated by the treating health worker. Research staff will work alongside clinical staff. All treatments will be provided free of cost by MSF; and will follow MSF clinical guidelines.

Sample management

Venous blood samples will be collected by research staff and stored at 2-8°C. Full blood count samples will be batched and run retrospectively within 24 hours off-site. Biochemical biomarker samples will be stored for a maximum of four hours prior to centrifugation. Within 24 hours, centrifuged plasma will be stored at -20°C or below without freeze-thaw. Sample aliquots will be thawed overnight at between 2-8°C and aliquoted at room temperature prior to assay performance. Samples will be batched and biochemical biomarkers will be quantitatively measured using an instrumented multi-analyte immunoassay on the Ella platform. suPAR will also be measured using a quantitative near-patient lateral flow assay.

Remaining samples will be transported to Dhaka and stored at -80°C at the icddr,b for use in future ethically-approved studies with similar aims. We have suggested 3 years as an appropriate storage period in Bangladesh, reflecting a very generic estimate of the utility of these samples within the existing pandemic, however this will ultimately be determined by the local ERC.

Additional ethical considerations

The study sites serve the Rohingya refugees, who are a highly vulnerable population. One site is based within camp 22 and is expected to enrol a nearly exclusively refugee population (noting that for participants from camp 22, the treatment/study facility is within 5 minutes walk from any point in the camp), while the other is accessible to a mix of host and refugee populations. There is no discrimination based on host or refugee status regarding treatment practices nor provision of care by MSF, which is provided entirely free of cost. Overall, we expect the refugee population to encompass approximately 70% of participants.

2. Patna, Bihar, India

Partner institute

All India Institute of Medical Sciences (AIIMS), Patna, Bihar

AIIMS Patna was established in 2012 with the aim to disperse and regionalise the vision and objectives of AIIMS, New Delhi, one of Asia's premier institutes for medical education, research and patient care. The objective of AIIMS, Patna since conception have been to provide highly specialist, quality care to low-income populations free of cost, and develop into a center of excellence for quality medical education and research. The hospital is managed directly by the central government and prior to COVID-19 was a 960 bedded hospital and medical college. At present the hospital has been restructured into a dedicated 400 bed COVID-19 hospital, accepting only COVID-19 positive patients.

Co-investigators

Dr Pragya Kumar (Associate Professor, Community Medicine, AIIMS, Patna)
Professor Sadhana Sharma (Head of Biochemistry Department, AIIMS, Patna)
Dr Sanjay Pandey (Head of Flu OPD, AIIMS)

Funding (for site costs)

MSF-OCBA

Rationale

India has recorded 3.8 million cases of COVID-19 as of 02 September 2020. Bihar with a population of about 124 million is India's poorest and most densely populated state. It has recorded over 140,000 COVID-19 cases and has ongoing community transmission.

With chronic resource constraints, dismal healthcare infrastructure and being a major source of economic and social migrants (skilled and unskilled labour) for the rest of the country, it has been expected that COVID-19 will overwhelm the already challenged healthcare capacities of the state. Besides COVID-19, the state has been ravaged with floods leading to internal displacement of over 2.5 million people, high malnutrition rates and overall lack of accountability within the healthcare sector, resulting in the state having one of the most vulnerable population groups in the country.

Site description

The study will be conducted at AIIMS, Patna located 20 km from the centre of Patna city. AIIMS, Patna was declared a dedicated COVID-19 hospital in July with a capacity of 400 beds. All

400 beds have an oxygen line, and 85 ventilators are available. The hospital has a dedicated Flu (SARI) clinic and acts as a referral centre, accepting only confirmed positive (RT-PCR or antigen) patients who are screened at the gate before entry to the Flu clinic. The clinic sees approximately 40 confirmed COVID-19 cases per day.

Enrolment procedures

MSF Research staff will be present in the Flu clinic of the study site and will work alongside the AIIMS Patna clinical staff. Screening and enrolment will occur after the patient has been assessed and initial management plan formulated by the treating doctor. No treating physician will have a role in the research, which will be performed by the MSF staff.

Sample management

Venous blood samples will be collected by research staff and stored at 2-8°C, and centrifuged on site. Full blood count samples and any COVID-19 swabs will be batched and run retrospectively within 24 hours off-site at the MSF Lab in Guru Gobind Singh Hospital. Biochemical biomarker samples will be stored for a maximum of four hours prior to centrifugation. Within 24 hours, centrifuged plasma will be stored at -20°C or below without freeze-thaw. Samples will be batched and biochemical biomarkers will be quantitatively measured using an instrumented multi-analyte immunoassay on the Ella platform. Remaining samples will be stored at -80°C at AIIMS for future approved studies.

Additional ethical considerations

AIIMS has been a long term and reliable research partner with MSF in previous studies that have been approved by the MSF ERB, which have been collaborations with the same PI (Dr Pragya Kumar). Prior to the conduct of any study activities the study will be approved by the Institutional Ethics Committee at AIIMS, Patna.

3. Chikungunya Clinical and Applied Research Network (REPLICK), Rio, Brazil

Partner institute

The National Institute of Infectious Diseases (INI)
Oswaldo Cruz Foundation (FIOCRUZ)
Chikungunya Clinical and Applied Research Network (REPLICK)

Co-investigators

Dr. Andre Siqueira (REPLICK network coordinator, FIOCRUZ/INI)
Dr. Julio Croda (FIOCRUZ/MS)
Dr. Guilherme de Sousa Ribeiro (FIOCRUZ/BA)
Dr. Luciano Pamplona (Federal University of Ceará)
Dr. Márcia Edilaine Lopes Consolaro (State University of Maringá)
Dr. Moacyr Jesus Barreto de Melo Rêgo (Federal University of Pernambuco)
Dr. Dhelio Batista Pereira (Tropical Medicine Research Center)
Dr. Fabio Juliano Negrão (Federal University of Grande Dourados)
Dr. Rodrigo Stabeli (FIOCRUZ/RB)

Funding (for site costs)

FIOCRUZ/INI

Rationale

Since the WHO announcement on December 31st 2019, COVID-19 has affected millions of people worldwide, and lead to the collapse of several health systems. In Brazil, the first confirmed case was registered on February 26th 2020 and since then, the number of cases has increased exponentially. The virus was detected in patients in all states of the federation, with a higher incidence in the southeast region. The disease has had major impact on Brazilian society with a significant increase in deaths and sequelae related to the disease, the impairment of the unified health system (SUS) and significant impact on the Brazilian economy with increases in social inequality. Understanding any question about the evolution of COVID-19 in the national territory is fundamental in order to propose more effective surveillance policies.

Site description

The Chikungunya Clinical and Applied Research Network (REPLICK) is coordinated by Dr. André Siqueira, headquartered at the National Institute of Infectious Diseases Evandro Chagas (INI-FIOCRUZ), and is responsible for conducting a multicenter study involving centers in nine different states, covering the 5 geographic regions of Brazil that represent the different scenarios in the country, encompassing the study of CHIKV infection. The REPLICK participating centers are fully operational under the guidelines of Good Clinical and Laboratory Practices, and the protocol

is being followed and monitored by the Ministry of Health in a pioneering experience of sharing data in real time with health authorities. REPLICK's experience has taken our local training to conduct clinical research, including planning clinical trials of therapeutics and preventive interventions that are being planned by the core team. For this proposal, we are taking advantage of this local and personal structure, already established since 2017, to assist the Multicentric Study of new coronavirus SARS-CoV-2 in Brazil.

This project will be conducted at REPLICK sites, that includes FIOCRUZ units from Rio de Janeiro (National Institute of Infection Disease), Salvador (Gonçalo Moniz Institute), Pernambuco, Campo Grande and Ribeirão-Preto, together with clinical research centers at the Federal University of Ceará, Pernambuco, Dourados and Mato Grosso do Sul, State University of Maringá (Paraná).

Enrolment procedures

The REPLICK team will be present in the health units in the different study sites for outpatients and hospitalised patients. Screening and enrolment will occur after patient has been assessed and initial management plan formulated by the treating health worker. All enrolment and follow-up procedures will be carried out in according to Section 7 of this protocol, after approval by the local ethics review board. In order to ensure the well-being and safety of the participants, the protocol will be conducting considering the Declaration of Helsinki and International Conference on Harmonisation and Good Clinical Practices (ICH/GCP).

Sample management

Relevant biological samples (including full blood counts and storage of plasma for retrospective measurement of biochemical biomarkers) are already being collected as part of ongoing REPLICK COVID-19 studies. This will continue and the laboratory processes are in alignment with Section 7 of this protocol. Where possible all laboratory assays will be performed within the REPLICK network. For any assays for which this is not possible (for example, due to unavailability of the necessary diagnostic platforms), samples will be shipped to a different laboratory (either within Brazil or overseas), subject to the necessary Material Transfer Agreements being signed and approval by the local ethics review board.

Additional ethical considerations

All participating centres must comply with the applicable Brazilian regulations for research involving human beings. The study will be conducted in accordance with the protocol, with the guidelines of the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP), and with the Document of the Americas, under resolution 441/2011 and resolution 466/2012 of the National Health Council.

The principal researcher of the coordinating centre (André Siqueira) is responsible for submitting the protocol, the ICF and other documents necessary for evaluation by the local ethics review board.

4. San Lazaro Hospital, Metro Manila, The Philippines

Partner institutes

San Lazaro Hospital (SLH)

Nagasaki University (NU)

Co-investigators

Dr. Rontgene Solante (Chair of Adult Internal Medicine and Tropical Medicine Department, SLH)

Dr. Ana Ria Sayo (Medical Specialist, SLH)

Dr. Chris Smith (Professor, Department of Tropical Medicine, NU)

Funding (for site costs)

UK Wellcome Trust and MSF-OCBA

Rationale

San Lazaro Hospital (SLH) is a public hospital with over 1,200 staff serving a mostly low-income population in the most densely populated area of Manila, the Philippines. The hospital manages patients with infectious diseases such as tuberculosis and HIV and has responded to large outbreaks of dengue and measles in recent years. SLH managed the first two COVID-19 cases in the Philippines and is a major COVID-19 testing and treatment centre.

Site description

SLH is a 500 bed public infectious diseases hospital located in Tondo, Manila and is a sub-national reference laboratory for COVID-19. There is an SLH-Nagasaki University research office and laboratory on-site and research staff comprising an office manager, post-doc, research nurses and lab technicians. As of September 2020, there were approximately 40 COVID-19 inpatients and 200-250 patients attending for testing per day.

Enrolment procedures

Research staff will be present in the triage (by the main entrance) and outpatient (screening tent located near the main entrance) locations of the hospital. Screening and enrolment will occur after patient has been assessed and initial management plan formulated by the treating health worker. Research staff will work alongside clinical staff. All treatments will be provided by SLH and will follow The Philippines national clinical guidelines.

Sample management

Sample storage and analysis will be undertaken in the on-site SLH-NU collaborative laboratory. Venous blood samples will be collected by research staff and stored at 2-8°C. Full blood count samples will be batched and run retrospectively within 24 hours.

Biochemical biomarker samples will be stored for a maximum of four hours prior to centrifugation. Within 24 hours, centrifuged plasma will be stored at -20°C or below without freeze-thaw. Sample aliquots will be thawed overnight at between 2-8°C and aliquoted at room temperature prior to assay performance. Samples will be batched and biochemical biomarkers will be quantitatively measured using an instrumented multi-analyte immunoassay on the Ella platform.

Remaining samples will be stored at -80°C at the SLH-NU lab for use in future ethically-approved studies with similar aims. We have suggested 3 years as an appropriate storage period in The Philippines, reflecting a very generic estimate of the utility of these samples within the existing pandemic, however this will ultimately be determined by the local ERC.

5. Christian Medical College, Vellore, India

Partner institute

Christian Medical College (CMC), Vellore, India

Co-investigators

Prof. George Varghese

Funding (for site costs)

UK Wellcome Trust and MSF-OCBA

Rationale

With healthcare facilities around India slowly being overwhelmed by the number of COVID-19 patients, there is a need for an effective and accurate way for predicting patients who have a low risk of progressing to require supplemental oxygen.

Site description

Christian Medical College (CMC) is a large teaching hospital that provides a range of primary to tertiary healthcare services for patients residing in Tamil Nadu and further afield from Southern India. The hospital provides care for over 2,000 inpatients and 8,000 outpatients daily across multiple sites. Since the COVID-19 outbreak the vast majority of hospital services have been directed towards this effort and the hospital has between 700-800 RT-PCR confirmed inpatients at any one time.

Enrolment procedures

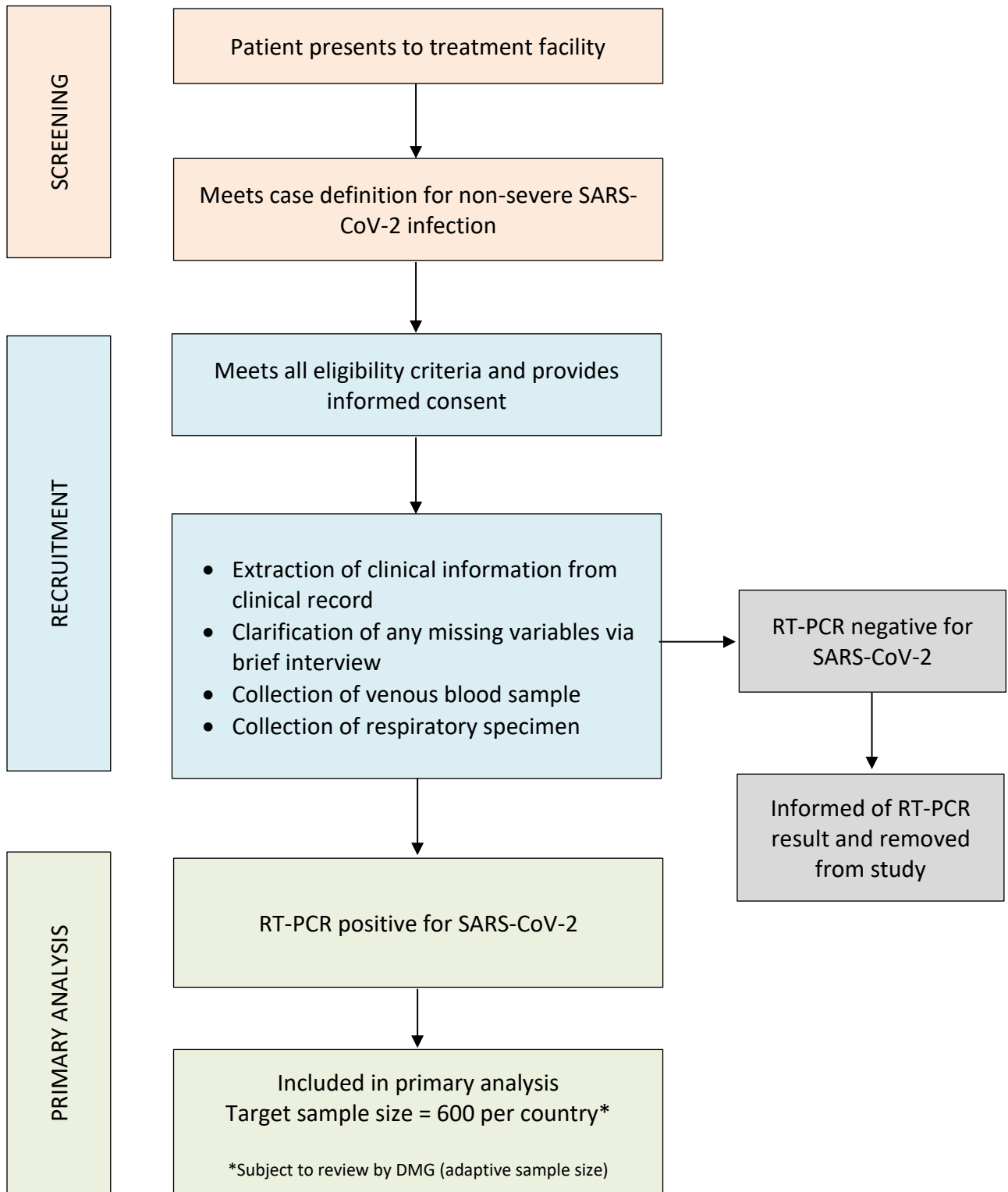
Research staff will be present in the triage and outpatient locations of the hospital. Screening and enrolment will occur after patient has been assessed and initial management plan formulated by the treating health worker. Research staff will work alongside clinical staff. All treatments will be provided by CMC and follow existing hospital clinical guidelines.

Sample management

Venous blood samples will be collected by research staff, stored at 2-8°C and centrifuged on site. Full blood count samples (that are not collected as part of routine care) will be batched and run retrospectively within 24 hours at CMC. Nasopharyngeal swabs (that are not collected as

part of routine care) will be batched and run retrospectively at CMC. Biochemical biomarker samples will be stored for a maximum of four hours prior to centrifugation. Within 24 hours, centrifuged plasma will be stored at -80°C without freeze-thaw. Samples will be batched and biochemical biomarkers will be quantitatively measured using an instrumented multi-analyte immunoassay on the Ella platform. Remaining samples will be stored at -80°C at CMC for future approved studies.

17. APPENDIX B: STUDY FLOW CHART



18. APPENDIX C: SCHEDULE OF STUDY PROCEDURES

Procedures	Visits		
	D0	D7 (+2)	D14 (+4)
Eligibility assessment	X		
Informed consent	X		
Extraction of information from clinical record	X	X	X
Venous blood sample (10 ml)	X		
Nasopharyngeal and/or oropharyngeal swab (study-specific procedure, if not collected as part of routine care)	X		
Assessment of vital status, symptom persistence and need for supplemental oxygen		X	X

19. APPENDIX D: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	3.0	07/09/20	Arjun CHANDNA	Protocol reformatted to provide site-specific details in Appendix A and describe 'over-arching' protocol in main document. Follow-up of patients who test negative for COVID-19 removed from protocol.
2	4.0	29/09/20	Arjun CHANDNA	Eligibility criteria modified. CMC, Vellore added to Appendix A.