EZ-FV-028

A cross-sectional, observational study to characterise Long-COVID in an urban sample of South African adults

Short Title:	ChaLoC
Protocol Version and Approval Date:	Version 02, 27 May 2022
Sponsor:	Ezintsha, Faculty of Health Sciences University of the Witwatersrand Building C, Sunnyside Office Park 32 Princess of Wales Terrance Parktown, Johannesburg 2193
Funder:	South African Medical Research Council
Principal Investigator:	Prof WD Francois Venter Ezintsha, Faculty of Health Sciences University of the Witwatersrand Building C, Sunnyside Office Park 32 Princess of Wales Terrace Parktown, Johannesburg, 2193

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

A cross-sectional, observational study to characterise Long-COVID in an urban sample of South African adults

I, the undersigned, am responsible for the conduct of the trial at this site and affirm that:

- I agree to personally conduct or supervise the study.
- I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined therein, as per any approved protocol amendments, as per ICH Good Clinical Practice (GCP) and all applicable national requirements and laws.
- I will not deviate from the protocol without prior review and written approval from the Institutional Review Board or Ethics Committee, except where necessary to prevent immediate danger to the participant.
- I will ensure that the requirements related to obtaining informed consent are in accordance with ICH Guidelines for GCP Section and local requirements.
- I agree to document adverse events that occur during the study, to maintain adequate and accurate records and make those records available, in accordance with ICH Guidelines for GCP, South African GCP and other local requirements. I agree to promptly report to the Ethics Committee all changes in the research activity and all unanticipated problems involving risk to the participants.
- I have sufficient time to properly conduct and complete the trial within the agreed trial period, and I will ensure that any qualified staff at my site(s) who are involved in the trial conduct are adequately trained regarding the protocol, and their responsibilities for the foreseen duration of the trial to conduct the trial properly and safely. If I delegate any of my trial activities, I will document this on a Delegation of Activities Form. I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
- I understand that the study may be terminated, or enrolment suspended at any time by me if it becomes necessary to protect the best interest of the participants.

Prof WD Francois Venter	Date

SPONSOR SIGNATORY APPROVAL PAGE

A cross-sectional, observational study to characterise Long-COVID in an urban sample of South African adults

I, the undersigned have read this protocol and I approve the design of this trial:

_____ _____

Nonkululeko Mashabane Date
Head of Research Operations
Ezintsha, University of the Witwatersrand

TABLE OF CONTENTS

ABBREVIATIONS AND TERMS

Term	Definition
ACTH	Adrenocorticotropic hormone
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CK-MB	Creatine kinase MB fraction
COVID-19	Coronavirus disease
CPM	Conditioned pain modulation
CST	Cosyntropin sensitivity test
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
cTnT	Cardiac troponin T
DLCO	Diffusing capacity of the lungs for carbon monoxide
DNA	Deoxyribonucleic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition)
ECG	Electrocardiogram
eCRF	Electronic case report form
EVDS	Electronic Vaccination Data System
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
HbA1C	Glycosylated haemoglobin
HIV	Human immunodeficiency virus
HREC	Human Research Ethics Committee
hs-CRP	High sensitivity C-reactive protein
IATA	International Air Transport Association
ICF	Informed consent form
ICU	Intensive care unit
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IL-8	Interleukin 8
LDH	Lactate dehydrogenase
Long-COVID	Post-COVID-19 syndrome / Post-acute sequelae of COVID-19 / Chronic COVID syndrome
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCP-1	Monocyte chemoattract protein-1
MCV	Mean cellular volume
MRC	Medical Research Council
MRI	Magnetic resonance imaging
OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction

POPIA	Protection of Personal Information Act
pro-BNP	Prohormone brain natriuretic peptide
PSG	Polysomnography
PTSD	Post-traumatic stress disorder
QST	Quantitative sensory testing
RDW	Red cell distribution width
SAMRC	South African Medical Research Council
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SBIMB	Sydney Brenner Institute for Molecular Bioscience
SpO_2	Peripheral oxygen saturation
TNF-α	Tumour necrosis factor alpha
TNFR1	Tumour necrosis factor alpha receptor 1
WHO	World Health Organization
WHO-DD	WHO Drug Dictionary
Wits	University of the Witwatersrand

SYNOPSIS

Title	A cross-sectional, observational study to characterise Long-COVID in an urban sample of South African adults
Short Title	ChaLoC
Background	"Long-COVID'' (also known as post-COVID-19 syndrome, post-acute sequelae of COVID-19, or chronic COVID syndrome, used here as 'Long-COVID' for brevity), is a complex array of postconvalescence symptoms following SARS-CoV-2 infection. The syndrome, common in COVID-19 survivors, can affect every organ system through as-yet uncharacterised but presumed immunological mechanisms. Prevalence depends on the definition used and time-period of follow-up, as well as the population being studied. The syndrome has been associated with significant and persistent disability in some survivors but has been hampered, until recently, by lack of a clinical definition, diagnostic criteria, and objective measures of disease or disability [1]. A Delphi-informed initial WHO clinical definition was released in early October 2021 but has attracted much criticism from both clinicians and survivors for a host of reasons, ranging from a lack of precision to a lack of inclusion [2]. Further complicating the syndrome is the context in which the SARS-CoV-2 epidemic occurred, which was associated with severe lockdowns in many countries (including South Africa) with social isolation, widespread fear and disinformation, widespread economic hardship, and loss of family and acquaintances, all of which contribute to symptoms (psychiatric and sleep disturbances, pain, and other syndromes) reported to be associated with Long-COVID. Finally, many Long-COVID symptoms overlap with those seen in patients hospitalised for any severe illness, especially those admitted to intensive care and ventilated. However, the proliferation of literature reporting associations of Long-COVID symptoms with more severe COVID-19 disease, and objective immunological, radiological, and organ-specific dysfunction in those reporting symptoms, suggests that the entity is real. The pathogenesis of Long-COVID is poorly understood, but this association with more severe disease - where immune dysregulation plays a major role in those with hospitalization, respiratory failure, and death - suggests an immune-mediated inflammatory dysfunction that may impact all organs [3-14]. The sheer rapidity of four major infection waves in South Africa, the initial focus on containing the hospital burden of those with severe illness, and subsequent emphasis on the roll-out of a mass vaccination program, has left little space for studying SARS-COV-2 sequalae in survivors. This group, loosely and inaccurately termed "recovered'' in South African reporting, were largely unvaccinated or partly vaccinated at the time of infection, leaving them at risk of developing Long-COVID. Long-COVID reported symptoms are extraordinarily broad. Over 50 symptoms were reported in a recent review, with over 80% of COVID-19 survivors having at least one symptom after infection [4]. The diagnosis is one of exclusion, and symptoms may fluctuate although tend to resolve with time. It is unclear whether disability and organ damage may be permanent in some patients. No specific treatment exists, and interventions are typically symptom-directed. SARS-CoV-2 vaccination appears to benefit many symptomatic survivors [15]. At the time of writing, no African data characterising the clinical features of Long-COVID have been published.
Rationale	Characterizing Long-COVID and related disability in local populations is an important first step to understanding the burden of morbidity and potential requirements of the health system in addressing symptoms and disability. Almost all studies on Long-COVID have been undertaken in higher income countries, and largely in hospitalised patients, with almost no data emerging yet from Africa. This study aims to characterise Long-COVID in South African patients by leveraging an existing

	patient base (including asymptomatic outpatients, symptomatic outpatients, hospitalised patients with severe COVID-19, and those who were vaccinated prior to widespread community exposure) and local research expertise. The study is anticipated to add significantly to understanding Long-COVID, and to contribute African data to current efforts to develop syndrome diagnostic criteria (including the WHO case definition) and an understanding of the therapeutic needs of symptomatic survivors, thus informing South African health care.
Design	This is a single centre, follow-up, observational, cross-sectional study of four distinct, longitudinal cohorts. Extensive clinical history will be obtained from each participant, and symptom questionnaire characterisation of Long-COVID (with a strong focus on organ-specific dysfunction, psychiatric, sleep, and pain parameters – all of which appear to be major features of Long-COVID), as well as laboratory and genetic characterisation will be performed. A subset of each cohort will be randomly selected for more specific syndrome characterisation related to sleep and pain, respiratory, cardiology, renal and glucose metabolism. The consequences of Long-COVID will be described and compared in four large, well-described clinical cohorts of African patients surviving SARS-CoV-2: • Cohort 1: asymptomatic subjects found to be PCR/antigen/antibody-positive during routine screening for SARS-CoV-2 infection • Cohort 2: symptomatic outpatients who were confirmed to have COVID-19 through a positive PCR/antigen test • Cohort 3: inpatients surviving hospitalisation for severe COVID-19 and who were PCR/antigen-positive • Cohort 4: participants vaccinated in clinical trials in 2020 prior to widespread community exposure to SARS-CoV-2, and hence protected from severe COVID-19 (and possibly Long-COVID) if subsequently infected. After obtaining informed consent from potential participants, a single cross-sectional, baseline visit will be conducted for each participant. Demographic data, clinical history (including COVID-19 history, targeted symptoms, and risk factors), COVID-19 vaccination dates (if administered), and details of previous and concomitant medications will be collected. Multiple questionnaires related to psychiatric screening, psychosocial factors, work function assessment, sleep quality, and pain assessment will be administered. Respiratory and cardiac function will be evaluated through a dyspnoea scale, walking test and an electrocardiogram (ECG). Laboratory evaluations will include a full blood count, serum chemistry, liver function tests, renal function assessment, inflammatory markers, and DNA extraction for genotyping. Blood and urine samples will be stored locally for possible future analysis. HIV testing will be performed for participants consenting to this optional assessment. After the baseline visit, participants with Long-COVID will be identified using the WHO clinical definition and general health assessments [2]. Randomly selected sub-groups of participants with, and without, Long-COVID will be selected from each of the four cohorts for additional investigations through participation in the following sub-studies: • Respiratory evaluation: dyspnoea assessment, high-resolution CT scan, lung function studies including spirometry and diffusion capacity (DLCO) • Cardiac evaluation: clinical history and examination, serial blood pressure, six minute walk test (distance), ECG, echocardiogram including speckle tracking, cardiac magnetic resonance imaging (MRI), creatine kinase MB fraction (CK-MB), cardiac troponin T (cTnT), prohormone brain natriuretic peptide (pro-BNP), and possible

	coronary angiography in patients with acute coronary syndromes and unstable angina • Psychiatric and neuroendocrine evaluation: questionnaires/surveys, semi-structured interview, home visit, saliva cortisol analysis, collection of diary data, actigraphy, adrenocorticotropic hormone (ACTH) challenge (cosyntropin sensitivity test [CST]), cellular immunity assessment • Sleep evaluation: questionnaires, actigraphy with sleep diaries, polysomnography • Pain evaluation: quantitative sensory testing (QST) and conditioned pain modulation (CMT) assessments • Glucose metabolism evaluation: oral glucose tolerance test (OGTT) including assessment of glucose, insulin, and c-peptide to estimate insulin sensitivity and beta-cell function [16]. Abnormalities detected in the assessments (including undiagnosed mental health issues) will be managed by on-study medical personnel with referral as appropriate.
Population	Adults of at least 18 years of age with previous confirmed SARS-CoV-2 infection (symptomatic or asymptomatic) will be invited to participate. The following eligibility criteria will be used to select study participants for the main study (baseline visit only): • Inclusion criteria (baseline visit/main study): 1. Able and willing to provide written or electronic informed consent for the baseline visit prior to any study-specific assessment or procedure. 2. Age at least 18 years at the time of signing the informed consent form. 3. Previous asymptomatic SARS-CoV-2 infection, confirmed through a documented positive PCR, antigen, or antibody test, at least six months prior to the baseline visit **[Cohort 1]** or, previous symptomatic SARS-CoV-2 infection for which hospitalisation was not required, confirmed through a documented positive PCR or antigen test at the time, at least six months prior to the baseline visit **[Cohort 2 only]** or, previous hospitalisation for management and treatment of COVID-19 confirmed through a documented positive PCR or antigen test at the time, at least six months prior to the baseline visit **[Cohort 3 only]** or, previous asymptomatic or symptomatic SARS-CoV-2 infection, confirmed through a documented positive PCR, antigen, or antibody test, at least six months prior to the baseline visit and received a COVID-19 vaccine in a non-placebo arm of a COVID-19 vaccine study during 2020 **[Cohort 4 only]**. 4. Willing to consent to verification of vaccination status on the national Electronic Vaccination Data System (EVDS). 5. Access to a reliable telephone or other device permitting information transfer. • Exclusion criteria (baseline visit/main study): 1. Symptomatic SARS-CoV-2 infection at any stage prior to the baseline visit **[Cohort 1 only]**. 2. Known SARS-CoV-2 infection, confirmed through a documented positive PCR test, prior to vaccination in a non-placebo arm of a COVID-19 vaccine study during 2020 **[Cohort 4 only]**. 3. COVID-19 within three months of the baseline visit. 4. Personnel (e.g., investigator, sub-investigator, research assistant, pharmacist, study coordinator or anyone mentioned in the delegation log) directly involved in the conduct of the study. 5. Any physical, mental, or social condition, that, in the Investigator's judgment, might interfere with the completion of the baseline assessments and

<table>
<tr><td></td><td colspan="2">evaluations. The Investigator should make this determination in consideration of the volunteer's medical history.
6. Participant is judged by the Investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.

Additional eligibility criteria will be used to identify study participants who are eligible for random selection for any of the sub-studies:
• Inclusion criteria (sub-studies):
1. Enrolled into main study and completed baseline visit.
2. Able and willing to provide written or electronic informed consent for the relevant sub-study.
• Exclusion criteria (sub-studies):
1. Pregnant women [Respiratory sub-study only].
2. Current or previous smokers [Respiratory sub-study only; refer to Respiratory Sub-Study Plan for definition of "previous"].
3. A well-characterised history of household exposure to biomass fuel used in the dwelling for heating and/or cooking, which preceded the acute COVID-19 illness [Respiratory sub-study only].
4. A well-characterised underlying comorbid respiratory illness being treated on a continuous basis, which preceded the acute COVID-19 illness [Respiratory sub-study only].
5. A well-characterised history of having had tuberculosis, appropriately diagnosed and treated, prior to the acute COVID-19 illness [Respiratory sub-study only].
6. A history of diabetes and/or use of treatment for diabetes mellitus (Type 1 or 2) prior to COVID-19 or asymptomatic SARS-CoV-2 infection [Glucose metabolism sub-study only].
7. Current, known diabetic or on treatment for diabetes mellitus (Type 1 or 2) [Glucose metabolism sub-study only].

Participants may be enrolled into more than one sub-study.</td></tr>
<tr><td>Treatment</td><td colspan="2">No treatment will be administered during this observational study.</td></tr>
<tr><td rowspan="2">Objectives and Endpoints</td><td>Primary Objective

To characterise Long-COVID in four cohorts of patients with previous SARS-CoV-2 infection (asymptomatic, symptomatic, severely symptomatic requiring hospitalisation, and those who were vaccinated prior to widespread community exposure).</td><td>Primary Endpoint

Incidence, severity, and duration of Long-COVID symptoms.</td></tr>
<tr><td>Secondary Objectives

To describe the following characteristics in four cohorts of patients with previous SARS-CoV-2 infection (asymptomatic, symptomatic, severely symptomatic requiring hospitalisation, and those who were vaccinated prior to widespread community exposure):</td><td>Secondary Endpoints</td></tr>
</table>

	• Inflammatory markers	• High sensitivity C-reactive protein (hs-CRP) • Interleukin-1β (IL-1β) • Interleukin-6 (IL-6) • Interleukin-8 (IL-8) • Tumour necrosis factor alpha (TNFα) • Tumour necrosis factor alpha receptor-1 (TNFR1) • Monocyte chemoattractant protein-1 (MCP-1)
	• Psychological profiles	• Headache Impact Test-6 • Patient Health Questionnaire-9 • Generalised Anxiety Disorder 7 • PTSD Checklist for DSM-5 - Civilian Version • Mood Disorder Questionnaire • Montreal Cognitive Assessment • Daily Fatigue Impact Scale
	• Psychosocial exposures	• COVID-19 related stress questionnaire • Multidimensional Scale of Perceived Social Support • Perceived Stress Scale • Adverse Childhood Experiences tool
	• Work performance in employed participants	• Normal activities and work productivity questionnaire
	• Sleep quality and disorders	• Pittsburgh Sleep Quality Index • Epworth Sleepiness Scale • Berlin Questionnaire for risk of sleep apnoea • International Restless Legs Syndrome Severity Scale • Sleep quality and mood visual analogue scale
	• Pain experience	• Brief Pain Inventory
	• Cardiorespiratory function	• Modified Medical Research Council Dyspnoea Scale • Six-minute walk test (distance) • ECG parameters and morphology
	• Standard laboratory parameters	• Full blood count • Serum chemistry • Liver function tests • Glucose, HbA1C
	• Renal function	• Creatinine clearance • Cystatin-C • Urine dipstick parameters • Urine albumin-to-creatinine ratio
	• Host genetic factors that may be associated with Long-COVID	• Genotyping results • DNA sequencing results

	Exploratory Objectives • The association between various specialised respiratory, cardiac, psychiatric and neuroendocrine, sleep and inflammation, pain, and glucose metabolism parameters and Long-COVID symptomatology will be explored in patients with Long-COVID from four cohorts of participants previously infected with SARS-CoV-2 compared to those with no signs or symptoms of Long-COVID (controls) from the same cohorts.
Sample Size	One hundred (100) participants will be enrolled into each cohort (400 participants overall). Enrolled participants with and without Long-COVID will be assessed for their suitability for various sub-studies. From the eligible participants, up to 30 from each cohort will be randomly selected to participate in each sub-study (while ensuring the requisite ratio of participants with Long-COVID to those without is maintained as applicable for each sub-study). The same participant may participate in more than one sub-study. No formal sample size calculation was performed for this observational study.
Duration	The duration of participation for each participant will either be a single visit (of approximately 6 hours), or two or more visits depending on whether they are enrolled into any sub-studies. Participants selected for the sleep study will have an overnight admission at the Wits Faculty of Health Sciences sleep laboratory. Enrolment to the study is expected to complete within one year.
Statistical Analysis	Participants enrolled in the main, and various sub-studies, will be summarised. Demographic data and other baseline characteristics will be summarised overall, per cohort, and per disease status (Long-COVID or no Long-COVID) as relevant. All primary and secondary endpoints will be summarised descriptively overall, per cohort, and per disease status (Long-COVID or no Long-COVID) as relevant, and for various risk factors as deemed appropriate in this exploratory study. Associations between outcomes, COVID-19 history, vaccination status prior to and since infection, and COVID-19 risk factors for severe disease will be explored. Co-factors such as time since initial SARS-CoV-2 infection, and possible confounders such as vaccination, exposure to potential disease-modifying repurposed drugs, and undisclosed vaccination or prior infections, will be accounted for as relevant. Raw genetic genotyping results will be quality controlled by experienced SBIMB bio-informaticists. Association and fine-mapping will be used to investigate links between host genetics and Long-COVID variables generated by the broader study.

1. SCHEDULE OF ASSESSMENTS

All participant enrolled will form part of the main study. Main study assessments, questionnaires, and procedures may be performed or administered in any order. Specific sub-study questionnaires and assessments are detailed in Sections 7.2 and in the sub-study-specific plans.

	Main Study			**Sub-Study(ies)**	
	Day 1 (+28[1])			**Day 2 to 180 (+28[1])**	
Procedure / assessment / questionnaire	**Screening**	**Baseline**	**FU#1**	**Sub-Study**	**FU#2**
Informed consent[2]	X				
SARS-CoV-2 infection and vaccination history[3]	X				
Main study eligibility review	X				
Demographic data		X			
Medical and surgical history[4]		X			
Long-COVID symptom-directed history[5]		X			
Previous and concomitant medications[6]		X			
Symptom-directed physical examination[7]		X			
Vital signs[8]		X			
Psychological and Somatic Symptoms of COVID-19 Survey		X			
Headache Impact Test-6 (HIT-6)		X			
Patient Health Questionnaire-9 (PHQ-9)		X			
Generalised Anxiety Disorder 7-item (GAD-7)		X			
PTSD Checklist – Civilian Version (PCL-C)		X			
Mood Disorder Questionnaire (MDQ)		X			
Montreal Cognitive Assessment (MOCA)		X			
Daily Fatigue Impact Scale (D-FIS)		X			
COVID-19 Related Stress Survey		X			
Multidimensional Scale of Perceived Social Support (MSPSS)		X			
Perceived Stress Scale-10 (PSS-10)		X			
Adverse Childhood Experiences (ACEs) Questionnaire		X			
Normal Activities and Work Productivity Questionnaire		X			
Pittsburgh Sleep Quality Index (PSQI)		X			
Epworth Sleepiness Scale (ESS)		X			
Berlin Questionnaire		X			
International Restless Legs Syndrome Study Group Severity Rating Scale (IRLS)		X			
Sleep quality and mood visual analogue scale		X			
Brief Pain Inventory (BPI)		X			
Modified MRC Dyspnoea Scale		X			
Six-minute walk test (distance)		X			
ECG[9]		X			
Blood sample for laboratory tests[10]		X			
Blood and urine sample storage[11]		X			
Optional HIV test		(X)			
Selection of participants for sub-study(ies)[12]		X----------	-----X		

	Main Study			Sub-Study(ies)	
	Day 1 (+28[1])			Day 2 to 180 (+28[1])	
Procedure / assessment / questionnaire	**Screening**	**Baseline**	**FU#1**	**Sub-Study**	**FU#2**
Telephonic follow-up			X[13]		X[14]
Sub-study informed consent				X[15]	
Participation in one, or more, sub-study				X----------X	
Collection of adverse event data[16]		X		X	

FU#1/2 = Telephonic follow-up number 1 or 2; ECG = electrocardiogram; HIV = human immunodeficiency virus

1 Time window for the follow-up telephonic contact is +28 days from completion of the assessments for the relevant part of the study.
2 Written or electronic informed consent to be provide prior to any screening or study-specific assessments, questionnaires or procedures are conducted.
3 Including dates of diagnosis through SARS-CoV-2 PCR or antigen testing, or date of positive antibody testing, COVID-19 vaccination dates (if administered), COVID-19 hospitalisation dates (if applicable).
4 Including information related to specific risk factors associated with poor COVID-19 outcomes and a detailed history of previous COVID-19 episodes (if applicable).
5 Targeting symptoms not covered in other administered surveys and questionnaires.
6 Details regarding previous and concomitant medications from the time of the participant's confirmed SARS-CoV-2 infection will be collected.
7 Including height and weight, and at least a general, cardiorespiratory, and skin examination and other systems as indicated through reported symptoms.
8 Vital signs (blood pressure, pulse rate, respiratory rate, body temperature, and SpO2) will be measured after 5 min resting.
9 A standard 12-lead ECG will be conducted after the participant has rested in the supine position for 10 minutes.
10 A blood sample will be collected for full blood count, serum chemistry, liver function, renal function, glucose, HbA1C, and inflammatory markers assessments, and for DNA extraction for genotyping and sequencing.
11 Blood (50 mL) and urine (100 mL) samples will be stored for possible future analysis pending emerging information.
12 Study team personnel will review each participant's baseline data and identify participants with and without Long-COVID who are eligible for participation in one, or more, sub-study. Invited participants will be randomly selected from the set of eligible participants for each sub-study. Participants who decline the invitation to participate in one, or more, sub-study, will be replaced by a randomly selected, matched-cohort participant where possible.
13 Participants will be contact telephonically to inform them of the results of their baseline assessments, and to arrange for follow-up clinical consultations as required for any identified medical or mental health issues. Participants that have been selected for sub-study participation will be invited to return for consent and enrolment into these sub-studies.
14 Participants will be contact telephonically to inform them of the results of their sub-study assessments, and to arrange for follow-up clinical consultations as required for any identified medical or mental health issues.
15 Written or electronic informed consent to be provide prior to any sub- study-specific assessments, questionnaires or procedures are conducted.
16 Adverse events associated with study assessments or procedures will be recorded in the eCRF for each participant.

2. INTRODUCTION

2.1 Background

"Long-COVID'' (also known as post-COVID-19 syndrome, post-acute sequelae of COVID-19, or chronic COVID syndrome, used here as 'Long-COVID' for brevity), is a complex array of postconvalescence symptoms following SARS-CoV-2 infection. The syndrome, common in COVID-19 survivors, can affect every organ system through as-yet uncharacterised but presumed immunological mechanisms. Prevalence depends on the definition used and time-period of follow-up, as well as the population being studied. The syndrome has been associated with significant and persistent disability in some survivors but has been hampered, until recently, by lack of a clinical definition, diagnostic criteria, and objective measures of disease or disability [1]. A Delphi-informed initial World Health Organisation (WHO) clinical definition was released in early October 2021 but has attracted much criticism from both clinicians and survivors for a host of reasons, ranging from a lack of precision to a lack of inclusion [2].

Further complicating the syndrome is the context in which the SARS-CoV-2 epidemic occurred, which was associated with severe lockdowns in many countries (including South Africa) with social isolation, widespread fear and disinformation, widespread economic hardship, and loss of family and acquaintances, all of which contribute to symptoms (psychiatric and sleep disturbances, pain, and other syndromes) reported to be associated with Long-COVID. Finally, many Long-COVID symptoms overlap with those seen in patients hospitalised for any severe illness, especially those admitted to intensive care and ventilated. However, the proliferation of literature reporting associations of Long-COVID symptoms with more severe COVID-19 disease, and objective immunological, radiological, and organ-specific dysfunction in those reporting symptoms, suggests that the entity is real. The pathogenesis of Long-COVID is poorly understood, but this association with more severe disease - where immune dysregulation plays a major role in those with hospitalization, respiratory failure, and death - suggests an immune-mediated inflammatory dysfunction that may impact all organs [3-14].

The sheer rapidity of four major infection waves in South Africa, the initial focus on containing the hospital burden of those with severe illness, and subsequent emphasis on the roll-out of a mass vaccination program, has left little space for studying SARS-COV-2 sequalae in survivors. This group, loosely and inaccurately termed “recovered’’ in South African reporting, were largely unvaccinated or partly vaccinated at the time of infection, leaving them at risk of developing Long-COVID.

2.2 Long-COVID

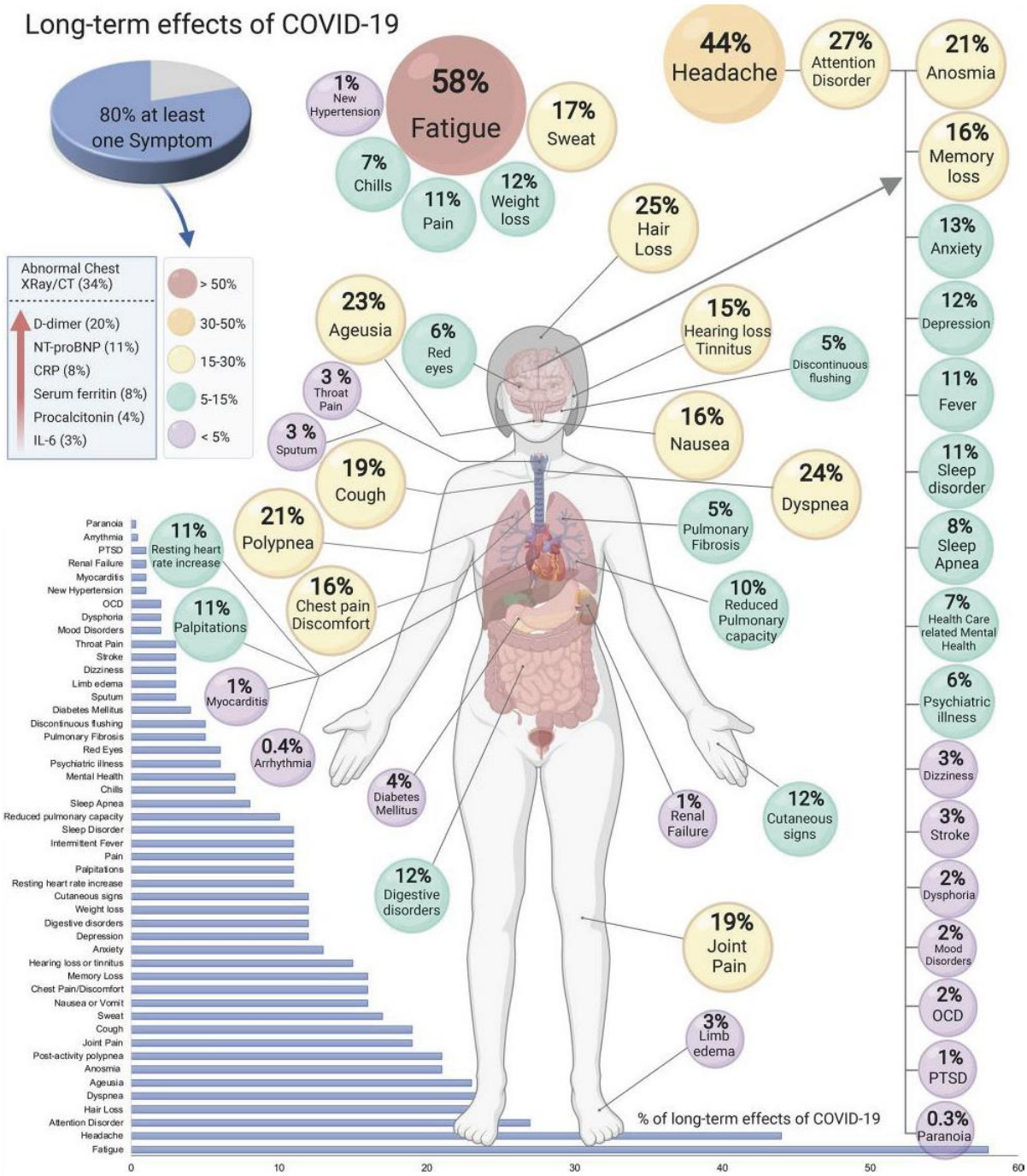

Figure 1: Long-term effects of coronavirus disease 2019 (COVID-19). The meta-analysis of the studies included an estimate for one symptom or more reported that 80% of the patients with COVID-19 have long-term symptoms.
CRP = C-reactive protein, CT = computed tomography, IL-6 = Interleukin-6, NT-proBNP = (NT)-pro hormone BNP, OCD = Obsessive Compulsive Disorder, PTSD = Post-traumatic stress disorder.
This figure was created using Biorender.com *(Reproduced under a Common Creation Licence, from Lopez-Leon, S., Wegman-Ostrosky, T., Perelman, C. et al [4])*

Long-COVID reported symptoms are extraordinarily broad. Over 50 symptoms were reported in a recent review, with over 80% of COVID-19 survivors having at least one symptom, sign, or abnormal

laboratory parameter after infection [4]. Fatigue (58%), headache (44%), attention disorder (27%), hair loss (25%), and dyspnoea (24%) were the most reported symptoms in this review, with abnormal chest x-ray or computed tomography (CT) scan findings detected in 34% of patients. Other symptoms and signs were related to lung disease (cough, chest discomfort, reduced pulmonary diffusing capacity, sleep apnoea, and pulmonary fibrosis), cardiovascular disorders (arrhythmias, myocarditis), neuropsychiatric diseases (dementia, depression, anxiety, obsessive–compulsive disorders), and others were non-specific such as tinnitus and night sweats.

The diagnosis of Long-COVID is one of exclusion, and symptoms may fluctuate although tend to resolve with time. It is unclear whether disability and organ damage may be permanent in some patients. No specific treatment exists, and interventions are typically symptom-directed. SARS-CoV-2 vaccination appears to benefit many symptomatic survivors [15].

At the time of writing, no African data characterising the clinical features of Long-COVID have been published.

2.3 Study Rationale

Characterizing Long-COVID and related disability in local populations is an important first step to understanding the burden of morbidity and potential requirements of the health system in addressing symptoms and disability.

Almost all studies on Long-COVID have been undertaken in higher income countries, and largely in hospitalised patients, with almost no data emerging yet from Africa. This study aims to characterise Long-COVID in South African patients by leveraging an existing patient base (including asymptomatic outpatients, symptomatic outpatients, and hospitalised patients with severe COVID-19) and local research expertise.

The study is anticipated to add significantly to understanding Long-COVID, and to contribute African data to current efforts to develop syndrome diagnostic criteria (including the WHO case definition) and an understanding of the therapeutic needs of symptomatic survivors, thus informing South African health care.

2.3.1 Rationale for specific sub-study focus areas

- Psychiatric, pain and sleep assessments: All are major features of reported Long-COVID and are associated with impact on quality of life [4,5,7,8,11]. Sleep disturbances are associated with chronic inflammation and immune activation.
- Respiratory assessment: SARS-CoV-2 is a respiratory virus, and most of the severe morbidity and mortality seen with COVID-19 is due to lung involvement. Respiratory symptoms, parenchymal, and functional abnormalities have been well-described and are common in survivors [9,13].
- Cardiac assessment: Significant myocarditis, both acute and chronic, has been described with COVID-19 infection, and concern expressed about long term effects on function [12,17].
- Renal assessment: Kidney injury is common with acute SARS-CoV-2 infection, and subclinical inflammation and injury may persist for many months, with progressive decline in kidney function [10].
- Glucose metabolism assessment: Diabetes is a strong risk factor for severe COVID-19 disease, as well as for Long-COVID and B-cell dysfunction, often irreversible, in survivors [11,18].
- Genetic assessment: SARS-CoV-2, COVID-19, and vaccination immune responses have notable clinical heterogeneity and interindividual differences, whether susceptibility to the virus, severity of COVID-19, or vaccine response, and may be partly ascribed to host genetic factors. Early host

genetic investigations have been limited to acute COVID-19 in European-ancestry individuals, with almost no attention to genetic correlations with Long-COVID [19,20,21].

3. OBJECTIVES AND ENDPOINTS

Primary	
Objectives	**Endpoints**
To characterise Long-COVID in four cohorts of patients with previous SARS-CoV-2 infection (asymptomatic, symptomatic, severely symptomatic requiring hospitalisation, and those who were vaccinated prior to widespread community exposure).	Incidence, severity, and duration of Long-COVID symptoms.
Secondary	
Objectives	**Endpoints**
To describe the following characteristics in four cohorts of patients with previous SARS-CoV-2 infection (asymptomatic, symptomatic, severely symptomatic requiring hospitalisation, and those who were vaccinated prior to widespread community exposure):	
• Inflammatory markers	• High sensitivity C-reactive protein (hs-CRP) • Interleukin-1β (IL-1β) • Interleukin-6 (IL-6) • Interleukin-8 (IL-8) • Tumour necrosis factor alpha (TNFα) • Tumour necrosis factor alpha receptor-1 (TNFR1) • Monocyte chemoattractant protein-1 (MCP-1)
• Psychological profiles	• Headache Impact Test-6 (HIT-6) • Patient Health Questionnaire-9 • Generalised Anxiety Disorder 7 • PTSD Checklist for DSM-5 - Civilian Version • Mood Disorder Questionnaire • Montreal Cognitive Assessment • Daily Fatigue Impact Scale
• Psychosocial exposures	• COVID-19 related stress questionnaire • Multidimensional Scale of Perceived Social Support • Perceived Stress Scale • Adverse Childhood Experiences tool
• Work performance in employed participants	• Normal activities and work productivity questionnaire

• Sleep quality and disorders	• Pittsburgh Sleep Quality Index • Epworth Sleepiness Scale • Berlin Questionnaire for risk of sleep apnoea • International Restless Legs Syndrome Severity Scale • Sleep quality and mood visual analogue scale
• Pain experience	• Brief Pain Inventory
• Cardiorespiratory function	• Modified Medical Research Council (mMRC) Dyspnoea Scale • Six-minute walk test (distance) • Electrocardiogram (ECG) parameters and morphology
• Standard laboratory parameters	• Full blood count • Serum chemistry • Liver function tests • Glucose, glycosylated haemoglobin (HbA1C)
• Renal function	• Creatinine clearance • Cystatin-C • Urine dipstick parameters • Urine albumin-to-creatinine ratio
• Host genetic factors that may be associated with Long-COVID	• Genotyping results • DNA sequencing results
Exploratory Objectives	
• Various specialised respiratory, cardiac, psychiatric and neuroendocrine, sleep and inflammation, pain, and glucose metabolism parameters will be explored in patients with Long-COVID from four cohorts of participants previously infected with SARS-CoV-2 compared to those with no signs or symptoms of Long-COVID (controls) from the same cohorts.	

4. STUDY DESIGN

4.1 Overall Design

This is a single-centre, follow-up, observational, cross-sectional study of four distinct, longitudinal cohorts. Extensive clinical history will be obtained from each participant, and symptom questionnaire characterisation of Long-COVID (with a strong focus on organ-specific dysfunction, psychiatric, sleep, and pain parameters – all of which appear to be major features of Long-COVID), as well as laboratory and genetic characterisation will be performed. A subset of each cohort will be randomly selected for more specific syndrome characterisation related to sleep and pain, respiratory, cardiology, renal and glucose metabolism.

The consequences of Long-COVID will be described and compared in four large, well-described clinical cohorts of African patients surviving SARS-CoV-2:

- Cohort 1: asymptomatic subjects found to be PCR/antigen/antibody-positive during routine screening for SARS-CoV-2 infection

- Cohort 2: symptomatic outpatients who were confirmed to have COVID-19 through a positive PCR/antigen test
- Cohort 3: inpatients surviving hospitalisation for severe COVID-19 and who were PCR/antigen-positive
- Cohort 4: participants vaccinated in clinical trials in 2020 prior to widespread community exposure, and hence protected from severe COVID-19 (and possibly Long-COVID) if subsequently infected.

After obtaining informed consent from potential participants, a single cross-sectional, baseline visit will be conducted for each participant. Demographic data, clinical history (including COVID-19 history, targeted symptoms, and risk factors), COVID-19 vaccination dates (if administered), and details of previous and concomitant medications will be collected. Multiple questionnaires related to psychiatric screening, psychosocial factors, work function assessment, sleep quality, and pain assessment will be administered. Respiratory and cardiac function will be evaluated through a dyspnoea scale, walking test and an ECG. Laboratory evaluations will include a full blood count, serum chemistry, liver function tests, renal function assessment, inflammatory markers, and DNA extraction for genotyping. Blood and urine samples will be stored locally for possible future analysis. Human immunodeficiency virus (HIV) testing will be performed for participants consenting to this optional assessment.

After the baseline visit, participants with Long-COVID will be identified using the WHO clinical definition and general health assessments [2]. Randomly selected sub-groups of participants with, and without, Long-COVID will be selected from each of the four cohorts for additional investigations through participation in the following sub-studies:

- Respiratory evaluation: dyspnoea assessment, high-resolution computed tomography (CT) scan, lung function studies including spirometry and diffusion capacity (DLCO) [Section 7.2.2.1]
- Cardiac evaluation: clinical history and examination, serial blood pressure, six minute walk test (distance), ECG, echocardiogram including speckle tracking, cardiac magnetic resonance imaging (MRI), creatine kinase MB fraction (CK-MB), cardiac troponin T (cTnT), prohormone brain natriuretic peptide (pro-BNP), and possible coronary angiography in patients with acute coronary syndromes and unstable angina [Section 7.2.2.2]
- Psychiatric and neuroendocrine evaluation: questionnaires/surveys, semi-structured interview, home visit, saliva cortisol analysis, collection of diary data, actigraphy, adrenocorticotropic hormone (ACTH) challenge (cosyntropin sensitivity test [CST]), cellular immunity assessment [Section 7.2.2.3]
- Sleep evaluation: questionnaires, actigraphy with sleep diaries, polysomnography (PSN) [Section 7.2.2.4]
- Pain evaluation: quantitative sensory testing (QST) and conditioned pain modulation (CPM) assessments [Section 7.2.2.5]
- Glucose metabolism evaluation: oral glucose tolerance test (OGTT) including assessment of glucose, insulin, and c-peptide to estimate insulin sensitivity and beta-cell function [16]. [Section 7.2.2.6]

Abnormalities detected in the assessments (including undiagnosed mental health issues) will be managed by on-study medical personnel with referral as appropriate.

4.2 Study Treatments

No treatment will be administered during this observational study. Participants taking medication(s) at the time of screening and enrolment, will continue to take this throughout their participation in the study.

4.3 Study Duration

The duration of participation for each participant will either be a single visit (of approximately 6 hours), or two or more visits depending on whether they are enrolled into any sub-studies.

Total duration for each participant will be up to 6 months depending on sub-study enrolment.

Participants selected for the sleep study will have an overnight admission at the University of the Witwatersrand (Wits) Faculty of Health Sciences sleep laboratory.

4.4 Individual Participant Withdrawal

A participant may be withdrawn from the study for any of the following reasons:

- At the request of the participant (withdrawal of informed consent), irrespective of the reason for this
- At the request of the primary care provider if he or she thinks the study is not in the best interest of the participant
- At the discretion of the Investigator if he or she believes that continuation in the study would be detrimental to the participant's well-being in any way, or
- At the discretion of the Institutional Review Board/Ethics Committee if they believe that continuation in the study would be detrimental to the participant's well-being in any way.

Participants will be considered withdrawn if they state an intention to withdraw, fail to return for visits, or are lost to follow-up for any other reason. For participants who are lost to follow-up, the Investigator will attempt to trace the participant, and will demonstrate "due diligence" by documenting all steps taken to contact the participant (e.g., dates of telephone calls, home visit, etc.) in the source documents.

If a participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such withdrawal of consent.

If a participant withdraws from the study, he or she may request destruction of any samples taken and not tested. This must be documented by the Investigator in the site study records.

Participants who withdraw or are withdrawn will not be replaced.

5. STUDY POPULATION

Adults at least 18 years of age with previous confirmed SARS-CoV-2 infection (symptomatic or asymptomatic) will be invited to participate. Approximately 400 participants will be enrolled.

5.1 Cohorts

The following well-described cohorts of participants will be enrolled (approximately 100 participants in each):

- Cohort 1: participants who were asymptomatic but were found to be SARS-CoV-2 PCR/antigen/antibody-positive during routine screening previously

- Cohort 2: participants who were previous outpatients with symptomatic COVID-19 confirmed through a positive SARS-CoV-2 PCR/antigen test
- Cohort 3: participants who were previous inpatients and survived hospitalisation for severe COVID-19 confirmed through a positive SARS-CoV-2 PCR/antigen test
- Cohort 4: participants who were previously infected with SARS-CoV-2 (symptomatic or asymptomatic) confirmed through a positive PCR/antigen/antibody test after being vaccinated in a non-placebo arm of a SARS-CoV-2 vaccine trial in 2020, with a vaccine demonstrated to be effective against severe COVID-19.

Participants enrolled in early vaccine trials were screened out if they were concurrently infected with SARS-CoV-2 as detected through a minimum of PCR testing. Given that they were only exposed to the first wave of COVID-19 infections in South Africa during which severe lock-down measures were in place, the probability of them being infected with SARS-CoV-2 prior to vaccination would have been low.

5.2 Recruitment

Potential participants may volunteer in response to advertised clinical trial information, or will be contacted in compliance with POPIA regulations and invited to participate in the informed consent process (Section 11.2) and attend the baseline visit. Written informed consent will be obtained prior to any screening, and study-specific assessments and procedures. The following platforms will be used to identify potential participants:

- Cohort 1:
    - Volunteers who had previous asymptomatic SARS-CoV-2 infection confirmed by a documented positive PCR/antigen/antibody test during routine screening (e.g. for travel, elective surgery or as part of contact tracing)
    - Participants enrolled into prophylaxis studies who had a documented PCR/antigen/antibody-positive test during routine screening but no symptoms of infection
    - Participants enrolled in self-sampling and antigen evaluation protocols with documentation of a positive PCR/antigen test during routine screening but no symptoms of infection.
- Cohort 2:
    - Volunteers who had previous symptomatic COVID-19 confirmed by a documented positive PCR/antigen test, and who were managed as outpatients
    - Participants enrolled in large, early treatment, antiviral studies in symptomatic outpatients
    - Participants enrolled into prophylaxis studies who developed symptoms of infection confirmed to be COVID-19 through a documented positive SARS-CoV-2 PCR/antigen test at the time
    - Participants enrolled in self-sampling and antigen evaluation protocols who developed symptoms of infection confirmed to be COVID-19 through a documented positive SARS-CoV-2 PCR/antigen test.
- Cohort 3:
    - Age- and gender-matched patients treated at the Helen Joseph Hospital and Charlotte Maxeke Johannesburg Academic Hospital Infectious Diseases department who were infected at approximately the same time as participants recruited for Cohorts 1 and 2.

- Cohort 4:
    - Participants enrolled in a non-placebo arm of a COVID-19 vaccine trial who received a vaccine that has subsequently been proven to be effective, and who were subsequently confirmed to be infected with SARS-CoV-2 (symptomatic or asymptomatic) through a documented positive PCR/antigen/antibody test.

5.3 Main Study Eligibility Criteria (Baseline Visit)

The following eligibility criteria will be used to select study participants for the main study (baseline visit only).

5.3.1 Inclusion criteria

1. Able and willing to provide written or electronic informed consent for the baseline visit prior to any study-specific assessment or procedure.
2. Age at least 18 years at the time of signing the informed consent form.
3. Previous asymptomatic SARS-CoV-2 infection, confirmed through a documented positive PCR, antigen, or antibody test, at least six months prior to the baseline visit **[Cohort 1 only]**
   or,
   previous symptomatic SARS-CoV-2 infection for which hospitalisation was not required, confirmed through a documented positive PCR or antigen test at the time, at least six months prior to the baseline visit **[Cohort 2 only]**
   or,
   previous hospitalisation for management and treatment of COVID-19 confirmed through a documented positive PCR or antigen test at the time, at least six months prior to the baseline visit **[Cohort 3 only]**
   or,
   previous asymptomatic or symptomatic SARS-CoV-2 infection, confirmed through a documented positive PCR, antigen, or antibody test, at least six months prior to the baseline visit and received a COVID-19 vaccine in a non-placebo arm of a COVID-19 vaccine study during 2020 **[Cohort 4 only]**.
4. Willing to consent to verification of vaccination status on the national Electronic Vaccination Data System (EVDS).
5. Access to a reliable telephone or other device permitting information transfer.

5.3.2 Exclusion criteria

1. Symptomatic SARS-CoV-2 infection at any stage prior to the baseline visit [Cohort 1 only].
2. SARS-CoV-2 infection, confirmed through a documented positive PCR, antigen, or antibody test, prior to vaccination in a non-placebo arm of a COVID-19 vaccine study during 2020 [Cohort 4 only].
3. COVID-19 within three months of the baseline visit.
4. Personnel (e.g., investigator, sub-investigator, research assistant, pharmacist, study coordinator or anyone mentioned in the delegation log) directly involved in the conduct of the study.
5. Any physical, mental, or social condition, that, in the Investigator's judgment, might interfere with the completion of the baseline assessments and evaluations. The Investigator should make this determination in consideration of the volunteer's medical history.

6. Participant is judged by the Investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.

5.4 Sub-Study Eligibility Criteria (Subsequent Optional Visit(s))

The following additional eligibility criteria will be used to identify study participants who are eligible for random selection for any of the sub-studies. Participants may be enrolled into more than one sub-study.

5.4.1 Inclusion criteria

1. Enrolled into main study and completed baseline visit.
2. Willing to provide written or electronic information consent for the relevant sub-study.

5.4.2 Exclusion criteria

1. Pregnant women **[Respiratory sub-study only]**.
2. Current or previous smokers **[Respiratory sub-study only**; refer to Respiratory Sub-Study Plan for definition of "previous"].
3. A well-characterised history of household exposure to biomass fuel used in the dwelling for heating and/or cooking, which preceded the acute COVID-19 illness **[Respiratory sub-study only]**.
4. A well-characterised underlying comorbid respiratory illness being treated on a continuous basis, which preceded the acute COVID-19 illness **[Respiratory sub-study only]**.
5. A well-characterised history of having had tuberculosis, appropriately diagnosed and treated, prior to the acute COVID-19 illness **[Respiratory sub-study only]**.
6. A history of diabetes and/or use of treatment for diabetes mellitus (Type 1 or 2) prior to COVID-19 or asymptomatic SARS-CoV-2 infection **[Glucose metabolism sub-study only]**.
7. Current, known diabetic or on treatment for diabetes mellitus (Type 1 or 2) **[Glucose metabolism sub-study only]**.

5.5 Justification of the Inclusion and Exclusion Criteria

The eligibility criteria have been selected to enable evaluation of participants with and without Long-COVID in clearly defined, real-life, cohorts with previous SARS-CoV-2 infection. Additional eligibility criteria have been selected to enable evaluation of specific organ systems in the context of Long-COVID in selected sub-groups of the enrolled cohorts.

5.6 Participant Identification

All volunteers who provide informed consent to participate in the study will be allocated a unique sequential screening number which will be used as the primary identifier for the duration of the study for enrolled participants.

5.7 Co-enrolment Guidelines

Participants may be co-enrolled in other research studies if these are observational in nature or include behavioural interventions only. Other co-enrolments require approval by the Principal

Investigator after consideration of possible confounding effects and participant safety and well-being regarding blood draw volumes and exposure to multiple assessments.

6. STUDY VISITS

A brief screening assessment will be conducted for all study participants who provide informed consent for participation in the study. Those who are eligible for enrolment will continue into a baseline visit where all main study questionnaires will be administered, and assessments and procedures conducted.

Participants who are eligible for various sub-studies will be identified by the study team after review of their baseline visit results. Sub-study participants (up to 30 per cohort per sub-study) will be selected from these eligible participants and will be invited to provide separate informed consent for each sub-study. An additional visit or visits will be conducted for each sub-study. The number of study visits for each participant will depend on the number and type of sub-studies in which they participate. All study visits (including the screening/baseline visits and sub-study visits) will be completed for each participant within approximately 6 months.

Participants will be contacted telephonically within 28 days of the baseline visit, and again within 28 days of their last sub-study visit (if relevant) to inform them of the results of their assessments. For participants taking part in the overnight sleep study, however, feedback may be delayed until 6 months from the overnight visit. Abnormalities detected in assessments (suggesting undiagnosed medical, mental health, or sleep issues) will be managed by on-study medical personnel with referral as appropriate.

Questionnaires to be administered, and assessments and procedures to be conducted at each study visit (main and sub-studies) are detailed in Section 1. Details regarding each questionnaire, assessment and procedure are described in Section 7.

7. STUDY ASSESSMENTS, QUESTIONNAIRES, AND PROCEDURES

No study-specific assessments will be performed, or information gathered, until the potential participant has given written, informed consent (Section 11.2) for screening assessments and (if found to be eligible) for study participation.

The timing of all assessments and procedures is detailed in the Schedule of Assessments (Section **Error! Reference source not found.**).

7.1 Main Study

Screening and baseline assessments are expected to be performed on the same day but may be spread over two or more separate days given the expected duration of the visit (approximately 6 hours).

7.1.1 Screening assessments (Day 1)

SARS-CoV-2 infection and vaccination history will be collected. This will include:

- dates of positive SARS-CoV-2 PCR, antigen, and/or antibody tests
- dates of symptomatic COVID-19
- date and duration of hospitalisation for COVID-19 (if relevant)
- date(s) of COVID-19 vaccine(s) administration.

Review of the main study eligibility criteria will be performed for all participants providing informed consent for the main study (baseline visit). Participants may be re-screened if they may later become eligible for participation (due to, e.g., age or interval between SARS-CoV-2 infection and screening).

7.1.2 Baseline assessments, questionnaires, and procedures (Day 1 [+7 days])

The following main study (baseline) evaluations will be performed. These may be performed in any sequential order during the baseline visit. All questionnaires, surveys and rating scales will be administered in accordance with the specific guidelines associated with validated versions of the tools and will be included in the Study Assessments Manual.

Participants will be required to fast for 8 hours (water permitted *ad libitum*) prior to the drawing of blood for serum glucose assessments. If additional baseline assessments are scheduled for the same day as the blood draw, this will be performed as one of the first procedures of the visit and participants will be given a meal at the research unit before proceeding with the remaining assessments.

7.1.2.1 Demographics, social background and habits data

The following will be collected:

- Sex, age, race, and country of origin
- Town of residence
- Marital status, occupation, and educational attainment
- Socioeconomic status, access to healthcare, financial support, and debt
- Household conditions (including home type, water facility, toilet facility, assets), number of people in household, number of rooms in household used for sleeping, and number of people in household who have had COVID-19
- Use of tobacco products, alcohol, and illicit/street drugs.

7.1.2.2 Medical and surgical history

In addition to general information related to past and current relevant medical conditions and surgical procedures, and menstrual cycle, pregnancy and lactation status (for female participants only), the following specific information will be collected:

- COVID-19 history (for participants with previous symptomatic infection(s)) including:
    - Symptoms experienced (including cough, fatigue, fever, malaise, shortness of breath, ageusia, anosmia, and other symptoms)
    - Whether or not the participant was pregnant at the time of SARS-CoV-2 infection (female participants only)
    - Whether or not the participant required supplemental oxygen at the time of COVID-19
    - Whether or not the participant required intensive care unit (ICU) admission for COVID-19 (Cohort 3 only)
    - Whether or not the participant required mechanical ventilation for COVID-19 (Cohort 3 only)
- Long-COVID symptom-directed history to gather information about symptoms not assessed through the detailed questionnaires and surveys listed in Sections 7.1.2.5 to 7.1.2.6.

7.1.2.3 Previous and concomitant medications

Details of previous and ongoing medications taken from the time of the participant's confirmed SARS-CoV-2 infection will be collected.

7.1.2.4 Physical examination and vital signs

Height and weight (as measured in light indoor clothing or underwear only, but without shoes) will be measured, and body mass index (BMI) derived.

A symptom-directed physical examination will be performed (including at least a general, cardiorespiratory, and skin examination and other systems as indicated through reported symptoms).

Vital signs (blood pressure, pulse rate, respiratory rate, body temperature, and oxygen saturation [SpO_2]) will be measured after 5 min resting.

7.1.2.5 Psychological/psychosocial/sleep/work function evaluation

The following questionnaires/surveys will be administered to screen for general health and psychological issues:

- Psychological and Somatic Symptoms of COVID-19 Survey: a questionnaire assessing psychological symptoms of COVID-19 sequelae including fatigue, brain fog, headache, muscle weakness, delusions, hallucinations, disorganized thoughts/behaviours, slurred speech, and somatic symptoms
- Headache Impact Test-6 (HIT-6): a tool for assessing the impact of headaches in patients' day-to-day lives, including six main domains affected by headaches (pain, social functioning, role functioning, cognitive functioning, vitality, and psychological stress) [22]
- Patient Health Questionnaire-9 (PHQ-9): a multipurpose instrument for screening, diagnosing, monitoring, and measuring the severity of depression [23]
- Generalised Anxiety Disorder 7-item (GAD-7): a short screening tool evaluating 7 symptoms of anxiety in adults [24]
- PTSD Checklist – Civilian Version (PCL-C): a 20-item self-report measure that assessed the 20 DMS-5 symptoms of PTSD [25]
- Mood Disorder Questionnaire (MDQ): a short screening tool evaluating the symptoms of bipolar disorder [26]
- Montreal Cognitive Assessment (MOCA): a cognitive screening test designed to accurately and quickly assess short term memory, visuospatial abilities, executive functions, attention, concentration and working memory, language, and orientation to time and place [27]
- Daily Fatigue Impact Scale (D-FIS): a survey tool that assesses physical, cognitive, and psychosocial dimensions of fatigue in everyday life [28].

Psychosocial factors will be evaluated through the:

- COVID-19 Related Stress Survey: a survey assessing psychosocial stress experienced during the COVID-19 pandemic and lockdown [29]
- Multidimensional Scale of Perceived Social Support (MSPSS): a brief research tool designed to measure perceptions of support from 3 sources – family, friends, and a significant other; the scale is comprised of a total of 12 items, with 4 items for each subscale [30]

- Perceived Stress Scale-10 (PSS-10): a survey that assesses the degree to which situations in one's life are appraised as stressful; items are designed to query how unpredictable, uncontrollable, and overloaded respondents find their lives [31]
- Adverse Childhood Experiences (ACEs) Questionnaire: a short questionnaire assessing presence or absence of a pre-defined list of childhood traumas from the ages of 0 to 18 years [32].

Work performance in participants who are employed will be evaluated using the:

- Normal Activities and Work Productivity Questionnaire.

Sleep quality and associated disorders will be evaluated using the:

- Pittsburgh Sleep Quality Index (PSQI): a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval [33]
- Epworth Sleepiness Scale (ESS): a self-administered questionnaire consisting of 8 questions used to assess daytime sleepiness [34]
- Berlin Questionnaire: a self-administered questionnaire designed to identify subjects at high risk for obstructive sleep apnoea [35]
- International Restless Legs Syndrome Study Group Severity Rating Scale (IRLS) [36]
- Visual analogue scale: 10 cm lines with 2 anchors will be used to assess sleep quality and mood (best sleep ever had – worse sleep ever had; feeling happy and motivated, wanting to do things – feeling down and low, not wanting to do things).

7.1.2.6 Pain assessment

Pain experience will be assessed using:

- The Brief Pain Inventory (BPI): a widely used questionnaire for the assessment of pain intensity, location, and interference with activities of daily living in clinical and research settings [37].

7.1.2.7 Cardiorespiratory assessment

Basic cardiorespiratory function will be evaluated using:

- The mMRC Dyspnoea Scale [38]
- A standard 12-lead ECG performed after 10 minutes resting in the supine position
- A six-minute walk test: a sub-maximal exercise test used to assess aerobic capacity and endurance [39].

7.1.2.8 Laboratory assessments

Blood sampling will be performed to collect samples for the following laboratory assessments:

- Full blood count: red cell count, haemoglobin, haematocrit, mean cellular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), white cell count and differential (neutrophil count, lymphocyte count, monocyte count, eosinophil count, and basophil count), and platelet count
- Serum chemistry: serum sodium, potassium, chloride, bicarbonate, calcium (corrected), magnesium, phosphate, urate

- Liver function: total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH)
- Renal function: urea, creatinine, estimated creatinine clearance (Cockcroft-Gault method), cystatin C
- Glucose metabolism: serum glucose, HbA1C
- Inflammatory markers: a multiplex cytokine panel will be run using Luminex technology at the Wits School of Physiology. Markers investigated will include hs-CRP, IL-1β, IL-6, IL-8, TNF-α, soluble TNFR1, and MCP-1, but additional inflammatory markers may be included based on emerging reports in scientific literature
- DNA extraction for genotyping and sequencing at the Sydney Brenner Institute for Molecular Bioscience (SBIMB)
- HIV testing for participants consenting to this (optional).

A urine sample will be collected for:

- urinalysis via urine dipstick
- determination of the urine albumin-to-creatinine ratio
- pregnancy test for woman of reproductive potential.

Additional blood (50 mL) and urine (100 mL) samples will be stored for possible future analysis.

Details regarding blood and urine sample collection, handling and processing, and the assays (where applicable) will be documented in the Laboratory Manual.

7.1.3 Telephonic follow-up (up to 28 days after completion of baseline visit)

Participants will be contacted telephonically within 28 days of completion of their baseline visit to inform them of the results of their assessments. Abnormalities detected in assessments (suggesting undiagnosed medical or mental health issues) will be managed by on-study medical personnel with referral as appropriate.

7.2 Sub-Studies

Participants may be invited to take part in more than one sub-study, or none. Up to 30 participants from each cohort will be enrolled into each sub-study. Participants with and without Long-COVID will be age- and gender-matched where possible.

7.2.1 Screening and informed consent

Separate informed consent will be obtained for each sub-study for participants invited to take part in these.

7.2.2 Sub-study visit(s)

7.2.2.1 Respiratory evaluation

Participants who are selected and provide consent for participation in the respiratory sub-study, will attend a single visit where the following will be performed:

- mMRC Dyspnoea Scale

- High-resolution CT scan of the chest
- A full set of lung function studies, including full spirometry and DLCO.

Depending on findings at this visit, participants may continue to be followed up for a further 3- to 6-month period.

Further details can be found in the Respiratory Sub-Study Plan.

7.2.2.2 Cardiac evaluation

Participants who are selected and provide consent for participation in the cardiac sub-study, will attend up to three additional visits where the following will be performed:

- Clinical history and examination
- Serial blood pressure measurements
- Six minute walk test (distance)
- ECG
- Echocardiogram including speckle tracking
- Cardiac MRI
- Blood sampling for:
    - CK-MB
    - cTnT
    - pro-BNP.

Coronary angiography may be performed at the discretion of the treating cardiologist and dependent upon the clinical setting in patients with a clinical indication for diagnostic coronary angiography (e.g., acute coronary syndromes, unstable angina).

Further details can be found in the Cardiac Sub-Study Plan.

7.2.2.3 Psychiatric and neuroendocrine evaluation

Participants who are selected and provide consent for participation in the psychiatric and neuroendocrine sub-study, will have the following additional assessments performed:

- Repeated administration of questionnaires related to the psychological impact of COVID-19
- 4-day home collection of cortisol samples, sleep measurements and diary data
    - Serial collection of passive drool saliva samples for evaluation of cortisol concentrations
    - Objective measurements of sleep timing and quality through a wearable, accelerometry device (actigraphy)
    - Morning and evening daily diary completion to collect data related to sleep quality, health behaviours and stressors
- ACTH challenge (CST)
- *Ex vivo* cell culture analysis to evaluate cellular immunity through assessment of leukocyte inflammatory responses to a microbial challenge
- Semi-structured interview to assess perceptions of, and experiences related to, COVID-19

- A home visit to further understand the lived experience of long-COVID.

Further details can be found in the Psychiatric and Neuroendocrine Sub-Study Plan.

7.2.2.4 Sleep evaluation

Participants who are selected and provide consent for participation in the sleep sub-study, will have the following additional assessments:

- Repeated administration of questionnaires related to sleep and psychosocial factors
- Objective measurements of sleep performed within 60 days after the baseline visit:
    - Actigraphy with sleep diary for a week-long period
    - Overnight polysomnography (PSG) at the Wits Faculty of Health Sciences Sleep Laboratory at the end of the week-long actigraphy period.

Further details can be found in the Sleep Sub-Study Plan.

7.2.2.5 Pain evaluation

Participants who are selected and provide consent for participation in the pain sub-study, will attend a single visit where the following will be performed:

- QST (using the protocol developed by the German Network for Neuropathic Pain [40,41])
- CMT (using the protocol published by Lie et. al., 2017 [42])

Further details can be found in the Pain Sub-Study Plan.

7.2.2.6 Glucose metabolism evaluation

Participants who are not known diabetics, are not on treatment for diabetes mellitus (Type 1 or 2), and who have no history of diabetes or receiving treatment for diabetes mellitus (Type 1 or 2) prior to COVID-19 or asymptomatic SARS-CoV-2 infection, will be randomly selected for participation in the glucose metabolism sub-study. Those who provide consent for participation will attend a single visit where an OGTT, including measures of glucose, insulin, and c-peptide to estimate insulin sensitivity and beta-cell function, will be performed [16].

Further details can be found in Glucose Metabolism Sub-Study Plan.

7.2.3 Telephonic follow-up

Participants taking part in sub-studies will be contacted telephonically within 28 days of completion of their last sub-study visit to inform them of the results of their assessments. The exception is participants taking part in the overnight sleep study for whom feedback may be delayed until 6 months from the overnight visit. Abnormalities detected in assessments (suggesting undiagnosed medical or mental health issues) will be managed by on-study medical personnel with referral as appropriate.

7.3 Biohazard Containment

Precautions will be employed by all personnel in the handling of blood and urine specimens collected during this study.

All biological specimens will be transported using packaging mandated by national and regional regulations. Details of these procedures will be described in the Laboratory Manual and will comply with relevant IATA Dangerous Goods Regulations.

8. SAFETY MONITORING

8.1 Responsibilities for Ensuring the Safety of Study Participants

8.1.1 Principal Investigator

The Principal Investigator has a personal responsibility to closely monitor study participants and an inherent authority to take whatever measures necessary to ensure their safety, including ensuring that procedures and expertise are available to cope with medical emergencies during the study.

8.1.2 Study Sponsor

The Sponsor has an institutional responsibility to ensure participant safety and undertakes to promptly notify the Wits Human Research Ethics Committee (HREC) of findings that could adversely affect the safety of participants included in the study, impact the conduct of the study, or alter the HREC's approval of, or favourable opinion to continue, the study.

8.1.3 Medical Monitor

The Medical Monitor will provide medical review during the execution of the study. This oversight will include the review of safety information and the provision of applicable recommendations to both the Investigator and the Sponsor. This review is intended to facilitate protection of the study participants. Medical review of data will be specified in the medical section of the Monitoring Plan to be finalised prior to enrolment of the first participant.

In addition to routine review of medical data, the Medical Monitor will also support the site and provide advice on protocol clarifications, assessment of eligibility and required medical follow-up of participants as and when indicated.

8.2 Adverse Events

8.2.1 Definitions

Some of the assessments and procedures used in this study may be associated with rare discomfort or adverse events (AEs), e.g., blood sampling, six-minute walk test, injection of contrast media, skin irritation from electrodes used in polysomnography.

For this study, AEs associated with study assessments or procedures will be recorded in the eCRF for each participant. The following information be recorded for each AE:

- a description of the AE
- the dates of onset and resolution of the event
- the characteristics of the event (seriousness, severity in accordance with CTCAE Version 5.0, Nov 2017 [43])
- the action taken in response to the event (including treatment required)
- the outcome of the event.

9. STATISTICAL CONSIDERATIONS

Details of the statistical analyses and their presentation will be documented in the Statistical Analysis Plan (SAP).

9.1 Sample Size Determination

No formal sample size was calculated for this exploratory study. On-study findings may suggest that larger sample sizes are required to further analyse endpoints in specific groups, and further planning for this may be performed.

9.2 Analysis and Presentation of Data

9.2.1 Disposition, demographic, and background data

Participants enrolled in the main, and various sub-studies, will be summarised. Demographic data and other baseline characteristics will be summarised overall, per cohort, and per disease status (Long-COVID or no Long-COVID) as relevant.

9.2.2 Primary and secondary endpoints

All primary and secondary endpoints will be summarised descriptively overall, per cohort, and per disease status (Long-COVID or no Long-COVID) as relevant, and for various risk factors as deemed appropriate in this exploratory study. Associations between outcomes, COVID-19 history, vaccination status prior to and since infection, and COVID-19 risk factors for severe disease will be explored. Co-factors such as time since initial SARS-CoV-2 infection, and possible confounders such as vaccination, exposure to potential disease-modifying repurposed drugs, and undisclosed vaccination or prior infections, will be accounted for as relevant.

Raw genetic genotyping results will be quality controlled by experienced SBIMB bio-informaticists. Association and fine-mapping will be used to investigate links between host genetics and Long-COVID variables generated by the broader study.

9.2.3 Safety data

All AEs will be listed.

9.2.4 Exploratory analyses

Details of exploratory endpoints and analyses to be performed will be included in the SAP.

10. STUDY MONITORING

Study conduct will be monitored by a research site monitor. Review of individual participant records, including consent forms, electronic case report forms (eCRFs), supporting data, questionnaire responses, and laboratory specimen records will be performed as detailed in the Clinical Monitoring Plan, to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect the site files to ensure that good clinical practice requirements are being followed.

The Clinical Monitoring Plan will describe these activities and will take into consideration necessary adaptations to be made if physical access to the site is limited at any stage due to pandemic restrictions.

11. ETHICAL CONSIDERATIONS

11.1 Ethical Considerations

The study will be conducted according to GCP (including South African GCP Guidelines [44]), the Belmont Report, the Declaration of Helsinki, and South Africa legal requirements regarding clinical research. The study protocol and relevant supporting documents will be submitted for review and approval by the Wits HREC responsible for oversight of research conducted at the study site. The study protocol will be registered with the South African National Clinical Trial Registry (www.sanctr.gov.za), National Human Research Ethics Committee (www.ethicsapp.co.za) and www.ClinicalTrial.gov. Six-monthly progress reports will be submitted to the HREC for the duration of the study, and as requested. Upon completion or premature termination of the study, the Investigator will provide the HREC with a summary of the study's outcome, and any reports required.

11.2 Informed Consent Process

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented before any protocol-specified procedures or interventions are carried out. The consent form will describe the purpose of the study, the assessments, questionnaires, and procedures to be performed, and the risks and benefits of participation. Separate consent forms will be available for the main study and all sub-studies. Participation in the sub-studies is voluntary and consent will be sought for these after completion of the baseline visit of the main study for participants who are eligible for further enrolment into sub-studies. Separate consent will be required for each sub-study in which a participant is enrolled.

HIV testing at the baseline visit is voluntary and will only be performed if participants provide explicit consent for this testing. Pre- and post-test counselling will be provided in accordance with local standard of care.

Potential participants will have the opportunity to have any questions answered before and after signing the informed consent forms (ICFs). The informed consent process and all questions raised will be documented.

The study staff who conduct the informed consent process will also sign the ICFs. A copy of the consent form(s) will be given to the participant, and this fact will be documented in the participant's record.

Any participant who is rescreened should be reconsented and eligibility for the study must be re-checked prior to enrolment.

11.3 Blood Volume

The volume of blood to be drawn from participants taking part in the main study is less than 100 mL. For a participant taking part in all additional sub-studies, the total blood volume across the entire study will not exceed 250 mL over 60 days.

11.4 Study Records and Confidentiality

The study site will establish a standard operating procedure for confidentiality protection. The site will ensure that study records, including ICFs, locator forms, case report forms, notations of all contacts with the participant, and all other source documents are stored in a secure manner.

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain participant confidentiality.

All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring, and auditing by the HREC.

The Principal Investigator or designee and all employees and co-workers involved with this study may not disclose or use, for any purpose other than performance of the study, any data, record, or other unpublished confidential information disclosed to those individuals for the purpose of the study.

All computers used during the study conduct will be password-protected, and records will only be accessible to authorised study staff.

11.5 Participant Remuneration

For each day of protocol-related study procedures, the participants will receive compensation for travel costs to and from the clinic and inconvenience incurred, as per local regulating body recommendations.

12. ADMINISTRATIVE CONSIDERATIONS

12.1 Protocol Amendments

Any protocol amendments will be prepared by the Sponsor and submitted to the HREC in accordance with their requirements.

Approval must be obtained from the HREC before the implementation of any changes, except for changes necessary to eliminate an immediate hazard to participants, or changes that involve logistical or administrative aspects only (e.g., change in contact information).

12.2 Clinical Data Records

A log of names, signatures and initials of all staff authorised to enter data into a participant's clinic file and eCRF will be kept.

The Investigator will maintain paper or electronic source documentation for all study participants. Protocol-specific participant information will be captured in an eCRF. The Clinical Data Management System will comply with guidelines and requirements for electronic systems used in clinical research.

Data validation and quality control procedures will be detailed in the Data Management Plan.

All deviations from this study protocol will be documented in the Trial Master File and included in the final study report. An assessment of the significance of each protocol deviation will be presented in the clinical study report.

12.3 Record Retention

All source data, clinical records and laboratory data relating to the study will be archived for a minimum period after completion of the study in accordance with South African GCP guidelines, Sponsor and Funder requirements. Data will be available for retrospective review or audit by arrangement with the appropriate representative at the archiving organisation (e.g., Sponsor Head).

12.4 Discontinuation of the Study

The Sponsor, the Funder, the Principal Investigator, and the HREC independently reserve the right to discontinue the study at any time for safety or other reasons. This will be done in consultation with the Funder, Sponsor and Principal Investigator where practical. In the event of premature termination or suspension of the study, the above-mentioned parties will be notified in writing by the

terminator/suspender stating the reasons for early termination or suspension. Following such a decision, the Sponsor and the Investigator will ensure that adequate consideration is given to the protection of the participants' interest and safety. Ongoing participants will be followed up telephonically to ensure that feedback related to the results of their assessments, questionnaires and procedures is provided.

12.5 Publication Policy

A dissemination plan will be developed with all project partners prior to study completion. After study completion, results will be disseminated using the following strategies: written methods (i.e., publications in peer reviewed scientific journals), presentations at scientific conferences and workshops, in person dissemination of results to the research participants, and using electronic methods such as the project website and electronic media to publish results.

12.6 Study Audits

Audits may be carried out by the SAMRC, Sponsor, or HREC quality assurance representatives. All documents pertinent to this study must be made available for such inspections after adequate notice of the intention to audit is provided.

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APPENDICES

APPENDIX 1: STUDY GOVERNANCE

The following Investigators and Institutional Affiliations are established. Designees may be provided, as appropriate.

Name	Role	Institution
Prof Francois Venter	Principal Investigator	Ezintsha, University of the Witwatersrand Building C Sunnyside Office Park 32 Princess of Wales Terrace Parktown, Johannesburg South Africa
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Dr Samanta Lalla-Edwards	Investigator (Research Lead)	