Master's in Medicine in Psychiatry: Dissertation

Persistent (>6 months) Neuropsychiatric symptoms in a cohort of SARS-CoV-2 PCR positive patients in Cape Town, South Africa

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Declaration

I, Inette van Niekerk, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise), and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. This work has not been reported or published prior to the registration for the above mentioned degree. This dissertation has been submitted to the Turnitin module and I confirm that my supervisor has seen the report.

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Format

This dissertation is being submitted in the format of a publication-ready manuscript which will be submitted to the journal *Brain, Behavior, and Immunity*.
The preliminary results of this study (excluding the O-link analysis) have been presented at the SASOP Biological Psychiatry Congress 2022.

Contributions of Authors

I van Niekerk conceptualized this study as part of her MMed in Psychiatry, under the supervision of A/Prof Jonny Peter and Prof Dan Stein. IvN wrote the research proposal and, with inputs from co-authors as described below, authored the manuscript of the publication-ready manuscript. After receiving feedback from examiners, IvN made corrections and re-worked the final manuscript.

Collaboration on this large project, the analysis, and the final manuscript, as described below:

T Müller and C Day recruited participants into the original ACE-2 and BCG vaccine studies

and collected clinical data at baseline and recovery visits for the parent studies. In addition, T Müller assisted with the compilation of the raw data into Excel spreadsheets and doing quality control; compiling selected parts of the Supplementary methods (study setting, severity, laboratory methods); compiling the sensitivity analysis; talking through some of the findings; and commenting on the draft. C Day made edits to and commented on the semi-final draft of the manuscript.

Data collection for the Post-Covid study was shared between IvN who interviewed half of the participants, and M Panieri who interviewed the other half of the participants.

IvN entered all the Post-Covid data onto REDCap for extraction by the data analysts.

S Dzanibe analysed the O-link, RAS, and antibody data and assisted with writing the methods of the O-link proteomic data; some parts of the supplementary methods pertaining to the O-link proteomic, RAS, and antibody assays; and talking through interpretations of the findings.

L Mapahla assisted with analysis of the post COVID-19 data using STATA software, compiling graphs, and compiling the statistical analysis section of the methods. L Mapahla compiled tables of baseline characteristics of the study sample.

IvN, TM, and CD were involved in data capture and cleaning.

JP supervised the study, assisted with interpretation of the findings and constructing the argument; assisted with co-ordination of the researchers; availability of facilities at the Lung Institute; and review of the final document.

DS co-supervised the study; reviewed the research proposal; advised on psychiatric aspects of the research and on choice of screening tools; and reviewed, made edits to, and commented on the final manuscript.

IvN was responsible for writing the manuscript, interpreting the findings of the various analyses and constructing the argument, reviewing the literature, co-ordinating the researchers, and compiling and editing the manuscript. IvN incorporated the comments and corrections

received from the Examiners into the final document and revised the final argument in the discussion, based on important recommendations made by Examiner 2.

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Abbreviations

ACE	Angiotensin Converting Enzyme
BBB	Blood-brain barrier
BCG	Bacillus Calmette-Guerin
C-SSRS	Columbia Suicide Severity Rating Scales
CFS-11	Chalder Fatigue Scale-11
CRP	C-reactive Protein
CSF	Cerebrospinal fluid
EHR	Electronic Health Records
FDR	False Discovery Rate

GAD-7	Generalized Anxiety Disorder Assessment-7
GSH	Groote Schuur Hospital
HIC	High Income Countries
ICU	Intensive Care Unit
IgG	Immunoglobulin G
KKS	Kallikrein-kinin system
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
LMIC	Low-and Middle Income Countries
LOD	Limit of detection
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NMDA	N-methyl-D-aspartate
NPX	Normalised Protein expression Scores
PASC	Post-Acute Sequelae of COVID-19
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
POPIA	Protection of Personal Information Act
PTSD	Post-Traumatic Stress Disorder
RAS	Renin-angiotensin-aldosterone system
SA	South Africa
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SNRI	Serotonin Norepinephrine Reuptake Inhibitors
SSRI	Selective Serotonin Reuptake Inhibitor
T-MoCA	Telephonic version of the Montreal Cognitive Assessment
UCT FHS	University of Cape Town Faculty of Health Sciences
WHO SRQ-20	World Health Organization Self-Report Questionnaire-20

Publication-ready manuscript prepared for *Brain, Behavior, and Immunity*

Persistent (>6 months) Neuropsychiatric symptoms in a cohort of SARS-CoV-2 PCR positive patients in Cape Town, South Africa

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Highlights

- More than half of participants from this South African post COVID-19 cohort were affected by neuropsychiatric symptoms of anxiety, fatigue, memory and cognitive impairment across the spectrum of disease severity (from asymptomatic to critical).
- Neither systemic inflammatory, cardiovascular, nor renin-angiotensin-system (RAS) biomarkers or antibody responses performed during the peak of illness or on a recovery sample at least three months after disease resolution, could predict patients with persistent neuropsychiatric symptom outcomes >6 months after infection.

Abstract

Background: SARS-CoV-2 is a neurotrophic and pro-inflammatory virus, with several acute and more persistent neuropsychiatric sequelae reported. There are limited data from younger African cohorts and few acute illness biomarkers of persistent neuropsychiatric symptoms.

Objectives: To determine the prevalence of neuropsychiatric symptoms in a cohort of South African SARS-CoV-2 PCR positive patients at least six months following infection/hospitalization. Second, to examine the association of neuropsychiatric outcomes with clinical illness severity, systemic inflammation, cardiovascular and renin-angiotensin-system (RAS) biomarkers.

Methodology: SARS-CoV-2 PCR positive patients were recruited prospectively from Cape Town, South Africa, including hospitalized patients from ancestral, beta and delta-dominant COVID-19 waves (pre-vaccine rollout); and asymptomatic/mild SARS-CoV-2 positive patients. Telephonic interviews were conducted at least six months post infection/hospitalization. Validated measures employed were: WHO Self-Report Questionnaire (SRQ-20), Generalized Anxiety Disorder Scale (GAD-7), Chalder Fatigue Scale (CFS-11) and Telephonic Montreal Cognitive Assessment (T-MoCA). The 96-protein O-link inflammation and cardiovascular panels, RAS fingerprinting, and antibody responses were measured in plasma/serum samples collected at peak severity and recovery (>3 months post-infection).

Results: Ninety-seven participants completed telephonic interviews. The median (IQR) age was 48 (37-59) years, and 54% were female. More than half of this South African COVID19 cohort had one or more persistent neuropsychiatric symptoms >6 months post vaccine-naïve infection. On the T-MoCA, 44% of participants showed evidence of cognitive and/or memory impairments. There were no significant associations between neuropsychiatric outcomes and illness severity, systemic inflammation, cardiovascular and renin-angiotensin-system (RAS) biomarkers.

Conclusion:. The high prevalence of persistent neuropsychiatric symptoms in this young African cohort supports ongoing attention to long COVID. Persistent neuropsychiatric outcomes post-COVID are not associated with systemic inflammation or altered renin-angiotensin physiology. Psychosocial variables affecting individual responses to the virus may explain the lack of association found on the biological front.

Keywords

Anxiety, biopsychosocial, cognitive impairment, fatigue, immunopathological, long COVID, long-term neuropsychiatric sequelae, memory, post-acute sequelae of COVID19 (PASC), post-COVID, renin-angiotensin system, SARS-CoV-2, serum immunoproteome

1. Introduction and background

The COVID-19 pandemic has significantly impacted the health of populations around the world. By 14 April 2024, South Africa has seen a total of 4.07 million confirmed cases and >102 595 deaths (1). SARS-CoV-2 has the potential to cause multisystemic complications, including neurological and neuropsychiatric manifestations, and also ongoing symptoms well beyond the acute infection (2, 3).

The World Health Organization's (4) standardised clinical case definition of the long COVID19 condition (also known as post COVID19 or post-acute sequelae of COVID19 [PASC]) (4) requires a history of probable or confirmed SARS-CoV-2 infection; with symptoms - which commonly include cognitive dysfunction, fatigue and shortness of breath - lasting more than or equal to three months from the onset of infection, and persisting for at least two months without an alternative diagnosis (4). The definition makes provision for new onset as well as persistent symptoms, and no minimum number of symptoms is required for the diagnosis (4).

SARS-CoV-2 is a neurotrophic virus, and in the post-pandemic period much attention has shifted to its medium and long-term neuropsychiatric sequelae. A systematic review and meta-analysis found that at least 45% of COVID19 survivors experienced at least one unresolved symptom (mean follow-up 126 days), with fatigue being the most prevalent (5). In a land-mark study of COVID19-related neuropsychiatric sequelae using large databases of Electronic Health Records from the United States of America (USA), Taquet et al retrospectively reviewed outcomes at the six-month and two-year marks post-infection, finding an estimated incidence of neurological or psychiatric diagnosis of 33.6%, with 12.8% receiving their first such diagnosis (6, 7). In the two-year follow-up of the larger retrospective cohort of 1, 284 437 patients, increased risk of psychotic disorders, cognitive deficit, dementia, and epilepsy or seizures persisted throughout the two-year study period (7). A comprehensive PASC symptom lexicon compiled from Electronic Health Records (EHRs) found that anxiety (25.8%), depression (24.0%), and fatigue (23.4%) were particularly prevalent (8).

There are currently no biomarkers in clinical practice able to predict long COVID19. Recent systematic reviews suggest both inflammatory (Interleukin 6 [IL-6], C-reactive protein [CRP] and Tumour necrosis factor-alpha [TNF-α]) and neuron-specific biomarkers (neurofilament

light chain [NFL] and glial fibrillary acidic protein) are increased in long COVID19 compared to both recovered or healthy control patients (9). Different biomarkers have been linked with different long COVID19 outcomes, with persistent elevation of chemokine CCL11 associated with cognitive dysfunction (10). Few studies have used unbiased approaches to examine acute or peak illness and recovery peripheral blood samples to determine whether biomarkers for persistent neuropsychiatric sequelae are evident during the acute stages of disease. Furthermore, the majority of biomarker studies have focused either on inflammatory/immune proteins or those linked to neuronal functioning; without considering the prominent cardiovascular, renin-angiotensin-aldosterone system (RAS) and kallikrein-kinin system (KKS) disturbances mediated by SARS-CoV-2 interactions with ACE2 receptors; despite genetic data linking the KKS pathway with anxiety (11).

There is a paucity of long COVID data from Lower Middle Income Countries (LMICs) (and in particular from Africa). Differences may exist between populations from high vs lower or middle-income countries especially pertaining to factors impacting on psychiatric symptoms and neurocognitive performance. These are influenced by several factors including age, education, infectious diseases and nutritional status (12). In South Africa, two studies were conducted which did not specifically focus on neuropsychiatric symptoms, but took a number of general and psychological symptoms into account. Dryden et al investigated long COVID19 at three months post-discharge, with two thirds of hospitalized COVID19 patients reporting new or persistent COVID19-related symptoms (13); while Jassat et al looked at persistent symptoms at the 6-month mark and found 46.7% of hospitalized, and 18.5% of non-hospitalized patients still experiencing symptoms (14). However, little is known regarding outcomes after 6-months in this setting.

New knowledge and treatment recommendations for post-COVID continue to evolve despite the many unanswered questions on pathophysiology (15). This strengthens the importance of this work and the argument for utility of screening patients for post-COVID neuropsychiatric symptoms.

Our study aimed to address several of these important research gaps. First, by determining the prevalence of persistent neuropsychiatric symptoms (>6 months post-infection/hospitalization) in a young cohort of South African SARS-CoV-2 PCR positive patients across the COVID19 disease severity spectrum. Second, to determine if demographic, clinical, and peripheral blood

markers including systemic inflammatory, cardiovascular disease, RAS fingerprinting and antibody responses were associated with these persistent neuropsychiatric outcomes.

2. Methods

2.1 Study setting, participants, and severity classification

A random selection of patients admitted with COVID19 (SARS-CoV-2 PCR positive) to Groote Schuur Hospital (GSH) were included as part of the UCT FHS COVID19 biorepository and ACE2 study enrolments. Admissions were from 21 June 2020 until 3 August 2021, across the first three waves of COVID-19 infections in South Africa (dominant variants: Ancestral, Beta and Delta) (16). From this multi-ethnic cohort, only one participant had received the COVID vaccine – one dose of the Pfizer vaccine – prior to natural infection. This participant was kept in our cohort, as the infection occurred soon after receiving the vaccination shot, meaning this participant would not have been fully protected by the single shot of vaccine yet. Adult non-pregnant patients with a positive SARS-CoV-2 PCR result were approached as early as possible following admission, with the main inclusion criteria being: ≤14 days post-admission, therefore acute samples were collected ≤14 days post symptom onset in all cases except for the asymptomatic cases. Asymptomatic cases were identified when blood was taken for other reasons and it was then discovered that these patients have had wild-type COVID antibodies indicating prior/current asymptomatic infection. Patients with pre-existing chronic kidney disease (CKD) were excluded. Data collected included: demographic, clinical, and laboratory data together with blood sampling at baseline and on average every three days until discharge or death; the median (range) number of samples was 4 (1-9) per patient. Details of GSH and the drainage population, or geographical area served by the hospital, are provided in the supplementary materials. A group of asymptomatic, non-hospitalized COVID19 participants from the same population were also included from the BCG Vaccine Study, full details of which are published (17). Telephonic contact and recovery serum sampling, wherever possible, were attempted for all discharged patients by the parent study researchers, starting from at least three months [range 3-17 months] post-discharge (44 % [99/224] of the total cohort were sampled).

Illness severity was scored using the WHO's COVID19 disease severity classification (18), with the only addition being evaluation of the ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) according to the National Institutes of Health (NIH) and US Food and Drug Administration (FDA) guidelines (19) (20). Since it proved important

to distinguish between ambulatory and hospitalized participants in analyses, SARS-CoV-2 infection severity was stratified into four groups according to the National Institute of Allergy and Infectious Diseases (NIAID) ordinal scale (21): Asymptomatic PCR-proven COVID19 infected; NIAID 1-2 (ambulatory, WHO mild-moderate disease); NIAID 3-5 (hospitalized, WHO moderate-severe disease, including PaO2/FiO2 $\leq$ 300 and >100); NIAID 6-7 (hospitalized with WHO critical disease or PaO2/FiO2 $\leq$ 100). The detailed methodology for severity classification is available in the supplement **(Table S1A and B)**. Additionally, the Western Cape Critical Care Triage Tool for decisions around ICU care and access to high-flow oxygen during peak surges is provided for reference.

2.2. Telephonic assessments of neuropsychiatric symptoms

All eligible participants were contacted and consented to telephonic assessment for persistent neuropsychiatric symptoms and completion of validated instruments. Telephonic interviews and questionnaires were administered by trained healthcare workers (psychiatry registrar and final year MD student). Interviews included the following self-report measures: Case report form; WHO Self-Report Questionnaire-20 (SRQ-20); Generalized Anxiety Disorder Assessment-7 (GAD-7); and Chalder Fatigue Scale-11 (CFS-11). For memory and cognitive symptoms, both subjective reports and objective screening was incorporated. The Telephonic version of the MoCA was administered to all participants who consented to this (93/97). The T-MoCA also divides participants into broad categories based on pre-morbid educational levels attained. This is important to detect whether broad differences in education between severity groups exist given that the majority of participants from the asymptomatic cohort were recruited from a different parent cohort (BCG Vaccine Study) than the mild-to-critical groups (ACE2-Study). Cognitive domains tested by the T-MoCA include learning and memory (including working memory, delayed recall and orientation), complex attention (digit span, vigilance/letter A tapping test, serial 7 subtractions, and orientation), and language (sentence repetition and phonemic fluency) (22, 23). Unlike the full version of the MoCA, the T-MoCA does not test executive function to the same degree, and it excludes questions that require visual cues or drawing (22). For the delayed recall domain, we also performed the category and multiple choice cue tests for each participant, which is an indication of whether any deficit is due to an encoding or retrieval problem, the former which does not improve with cueing, and the latter which does (23). The GAD-7 and CFS-11 have been recommended for the study of patients with COVID19 (24), and the SRQ-20 and GAD-7 have been validated for use in

LMICs (25). The Case report form included questions about pre-COVID mental health status (whether participants had pre-existing symptoms, mental health diagnoses, or received psychotropic medication or psychological treatment) and family history of mental illness. Further discussion of the use and suitability of each chosen instrument in this study setting is provided in the **supplementary methods**.

2.3 Routine and specialised laboratory data and methods

Participants enrolled in the context of the acute UCT FHS COVID19 biorepository did not have protocol specified blood sampling or laboratory testing; however standard-of-care at GSH for COVID19 in-patients (26) meant that basic blood parameters available for this cohort include baseline full blood counts, electrolytes and renal function, D-dimer and C-reactive protein (CRP) at least at admission. Specialised sampling was conducted for physiological measurements of RAS and is detailed in the supplementary methods. Serum samples were collected for each time-point and used for the O-link proteomic panels. The inflammatory and cardiovascular panels each consists of 96 proteins in total, yet analytes where the majority of the participants (>50%) had values below the limit of detection were excluded from further downstream analysis. Thus, our final analyses included: O-link inflammatory (75 markers) and cardiovascular (24 markers, 3 overlapping with inflammatory panel) proteomic panels. For the RAS analysis, markers were profiled in serum using both RAS fingerprinting via mass spectrometry (LC-MS/MS), as well as via enzymatic measurement of serum ACE1 and ACE2 activity (27).

Serum samples were selected for O-link analysis as more aliquots were available in the biobank. The difference between plasma and serum sampling is that clotting factors are removed from serum, yet it still contains all the antibodies (28). The question of whether plasma is superior to serum is still up for debate. Serum is subjected to ex vivo processing steps during which proteins can be released from platelets, leucocytes, and the clot itself, while some argue that plasma sampling resembles in vivo physiology more closely (28). Our study measured immunoglobulins which are thought not to be susceptible to ex vivo degradation (28). Furthermore, we looked at the O-link PEA for the inflammatory and cardiovascular panels and did not note major differences in prevalent proteins. O-link themselves did a comparative analysis and had not noted major differences either. In line with this, authors arguing for the

superiority of plasma sampling admit that their study results may not be generalizable if different proteomic assays are used (28).

Anti-nucleocapsid and anti-spike IgG measurements, as well as RAS fingerprinting was performed for two to three samples per participant which included matched peak illness severity and a recovery time-point at least 3 months post SARS-CoV-2 PCR (median time 4.5 months [range 3-17 months]).

The full detailed laboratory methods are provided in the supplementary materials.

2.4 Statistical analysis

Data were entered and stored on a secure online Research Electronic Data Capture repository (REDCap, version 12.0.19, Vanderbilt University, Nashville, Tenn.). Data were then de-identified and extracted into excel sheets for analysis. While the clinical data after initial infection were collected at baseline and at recovery from acute illness, the post-COVID neuropsychiatric questionnaires were done at a single time-point to assess persistent symptoms. Continuous variables were described using median and interquartile range (IQR) if not normally distributed or else mean and standard deviation was used while frequencies and proportions were used to describe categorical variables. Demographics, symptoms, comorbidities, disease severity, preceding and post-COVID mental health, and results of questionnaires were presented in frequency distribution tables and graphs. We used the chi square test of association to test the independence between categorical variables while the t-test of mean difference by a categorical variable was done for continuous variables. False discovery rates were used for corrections.

Statistical software used for these analyses included: STATA 15.1, R (29) (29) (30) and Microsoft excel (version 16.77, Redmond, Washington) (31). Data was captured and cleaned in Microsoft excel and then exported either to Stata 15.1 or R software for coding, statistical analysis, and visual presentations. All statistical tests were done at 5% level of significance. False discovery rates were used for corrections of t-tests. Continuous data are summarised as medians and interquartile ranges (IQR) and statistical differences tested between groups using Wilcoxon rank test and across multiple groups using Kruskal-Wallis test. Categorical data are

summarised as frequencies and statistical differences tested using Chi-squared or Fisher-exact test.

Olink-proteomic data

Patients' plasma proteomics were quantified using the O-link proximity extension assay and presented as $\log_2$ normalized protein expression scores (NPX). A total of 96 proteins from the Inflammation and Cardiovascular II panels were measured, and those whose NPX values were either missing or below the limit of detection (LOD) in >50% of the samples were excluded from further analysis (supplementary Table S2). The remaining 96 protein analytes whose NPX values were below LOD were either replaced with $\sqrt{LOD}$ or imputed using maximum-likelihood estimates form the nrom2 R package. Principal components analysis (PCA) of the protein NPX scores for each sample was performed using the prcomp R function for dimensional reduction and visualized using the ggplot2 R package. Quantile scaled values of protein NPX scores, RAAS and antibody titres for each study participant was visualised using a heatmap from the Complex Heatmap R package. Pairwise comparisons were performed using Wilcoxon rank test and computed p-values were corrected for multiple comparisons using false discovery rates (FDR). Adjusted $p<0.05$ was considered statistically significant.

2.5. Ethics and study participant clinical care

The parent studies namely BCG Vaccine study (Reducing morbidity and mortality in healthcare and other frontline workers at risk of exposure to SARS-CoV-2 by enhancing non-specific immune responses through Bacillus Calmette-Guerin vaccination, a double-blinded, randomized controlled trial) and ACE2 study (ACE2 and the renin angiotensin system as a COVID19 outcome predictor: activity and genetic variation in South African cohorts), as well as the present study, were approved by the University of Cape Town Health Science Faculty Research Ethics Committee (HREC R021/2020; 237/2020, and 711/2021). In accordance with the Protection of Personal Information Act (POPI Act), samples were anonymised and labelled with random study identifiers. Collected data were de-identified for analyses by using a separate password protected database linking patient information and study identifiers. Any participant answering in the affirmative to questions about suicidal thoughts on the SRQ-20 also completed the Columbia Suicide Severity Rating Scales (C-SSRS). At risk participants

who screened positive with severe symptoms were referred to local Mental Health Services for further assessment and management in accordance with our study protocol.

3. Results

3.1 Baseline participant characteristics

The consort diagram in Figure 1 outlines the cohort of patients included in this post-COVID study, listing the reasons for participant exclusions. In total, telephonic interviews were conducted for 99 participants. One of these were excluded due to language barrier affecting results, and another due to brain cancer, leaving a total of 97 study participants. Key reasons for no interview included: death (n=31), being uncontactable (n=60) or declining to have further sampling or telephonic interviews (n=32). There were no differences in demographic variables between participants from the overall ACE-2 cohort who were included and those who were not included in the post-COVID study, indicating a low likelihood for selection bias.

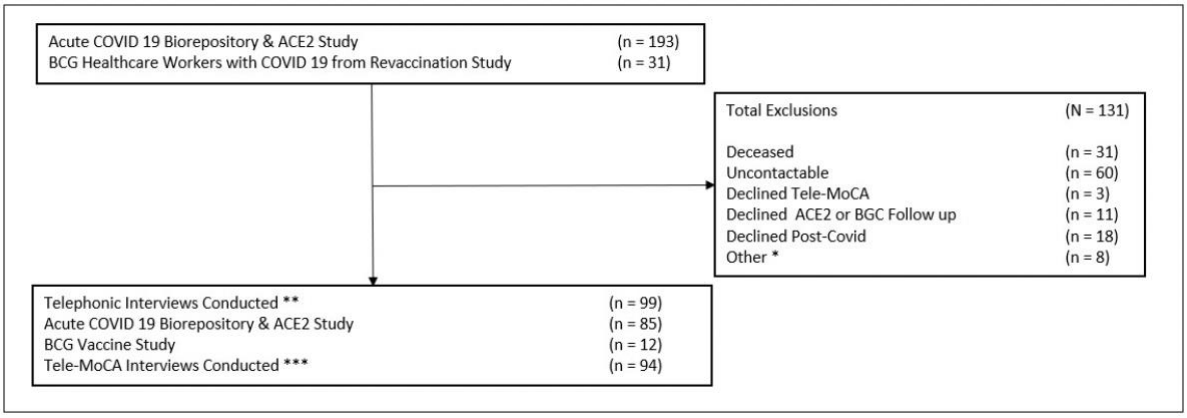


Figure 1: Consort diagram – total final participants n=97; total final T-MoCA n=93

* Other: Excluded due to language barrier (n=4), colleagues in the same profession (n=3) and brain cancer (n=1)

** 99 full telephonic interviews completed, whereafter one excluded due to language barrier and one excluded due to brain cancer

*** 94 T-MoCA conducted due to 3/97 participants declining T-MoCA. Final included T-MoCA n=93 due to a further participant excluded (reason: language barrier affecting T-MoCA results)

ACE2 = Angiotensin-converting enzyme 2

BCG = Bacillus Calmette-Guerin

T-MoCA = Telephonic Montreal Cognitive Assessment

Table 1: Baseline characteristics of interviewed patients stratified by neuropsychiatric outcomes

Characteristics	Complete Cohort, n=97	T-MoCA[a] n=93		Subj[b] n=97		GAD[c] n=97		Fatigue[d] N=97	
		Unimpaired, n=52	Impaired, n= 41	Unreported, n=46	Reported, n=51	Negative, n=F= 73	Positive, n=24	Negative, n=43	Positive, n=54
Age, mean(sd)	48 (11)	47 (12)	50 (10)	46 (12)	49 (11)	48 (12)	45 (9)	47 (12.00)	48 (11)
Female, x(%)	52 (54)	25 (48)	25 (61)	24 (52)	28 (55)	38 (52)	14 (58)	24 (56)	28 (52)
Duration of symptoms prior to baseline sampling, days med(IQR)	7 (5 – 11)	7 (5 – 11)	8 (5 – 11)	8 (5 – 12)	7 (5 – 10)	7 (5-11)	7.5 (5 – 11)	7.5 (5 – 11)	7 (5 – 11)
Symptoms, n(%)									
- Cough	45 (46)	25 (48)	18 (44)	23 (50)	22 (43)	32 (44)	13 (54)	17 (40)	28 (52)
- Fever	16 (32)	10 (19)	6 (29)	8 (17)	8 (32)	12 (32)	4 (17)	8 (19)	8 (29)
- Myalgia	6 (32)	5 (10)	1 (33)	1 (33)	5 (10)	4 (29)	2 (8)	2 (29)	4 (32)
- Headache	6 (32)	4 (8)	2 (29)	3 (32)	3 (32)	2 (33)[e]	4 (17)[e]	2 (29)	4 (32)
- GIT symptoms	6 (32)	3 (32)	3 (32)	4 (9)	2 (29)	5 (32)	1 (29)	4 (9)	2 (29)
PaO2/FiO2, med(IQR)	157 (74 – 270)	157 (74 – 252)	202 (75 – 277)	75 (56 – 269)	181 (83 – 270)	142 (70.5 – 246)	211 (77 - 310)	175 (56 – 277)	150 (77 – 252)
High flow nasal oxygen, x(%)	4 (29)	2 (29)	2 (32)	2 (29)	2 (29)	1 (33)	3 (14)	2 (29)	2 (29)
Duration of admission, med(IQR)	3 (2 – 5)	3 (2 - 5)	2 (2 - 4)	3 (2 – 5)	2 (2 – 4)	3 (2 – 5)	2.5 (1 – 5)	2 (2 – 5)	3 (2 – 5)
Hypertension, x(%)	38 (39)	19 (37)	19 (46)	15 (33)	23 (45)	31 (42)	7 (29)	15 (35)	23 (43)

Diabetes Mellitus type 2, x(%)	27 (28)	13 (34)	13 (32)	11 (24)	16 (31)	21 (29)	6 (34)	12 (28)	15 (28)
HIV, x(%)	4 (29)	2 (29)	2 (29)	1 (33)	3 (32)	3 (29)	1 (29)	0 (33)	4 (32)
White cell count, (10^9/L), med(IQR)	9 (7 – 11)	9 (– 13)	9 (7 – 10)	10 (7 – 12)	9 (7 – 11)	9 (7 – 13)	9 (6 – 10)	9 (7 – 13)	9 (7 – 10)
Lymphocyte count, (10^9/L), med(IQR)	1 (0.7 – 1.5)	1.3 (0.8 – 1.6)	1 (0.9 – 1.5)	1.2 (0.7 – 1.4)	1.1 (0.8 – 1.8)	1.2 (0.8 – 1.5)	0.9 (0.6 – 1.7)	1.2 (0.7 – 1.5)	1 (0.7 – 1.5)
Haemoglobin, (g/dL), med(IQR)	14 (13 – 14)	14 (13 – 14)	13 (13 – 15)	13 (12 – 14)	14 (13 – 15)	13 (13 – 14)	14 (13 – 14)	13 (12 – 14)	14 (13 – 14)
D-dimer, (mg/L), med(IQR)	0.4 (0.3 – 0.7)	0.4 (0.3 – 0.9)	0.4 (0.2 – 0.7)	0.4 (0.3 – 0.7)	0.4 (0.3 – 0.7)	0.4 (0.4 – 0.8)	0.3 (0.2 – 0.4)	0.4 (0.4 – 0.8)	0.4 (0.3 – 0.7)
C-reactive protein, (mg/L), med(IQR)	102 (62 - 150)	77 (45 – 131)	107 (70 – 152.5)	69 (38 – 93)[a]	122 (77 – 169)[a]	102 (62 – 169)	102 (53 – 127)	70 (45 – 104)	116 (74 – 169)
Creatinine, (mmol/L), med(IQR)	74 (66 – 89)	79 (69 – 91)	72 (66 – 88.5)	77 (66 – 93)	73 (66 – 89)[d]	77 (69 – 94)	69 (66 – 82)	77 (67 – 92)	71 (66 – 89)
Urea, (umol/L), med(IQR)	5 (4. – 7)	5 (4 – 7)	6 (4 - 7)	5 (4 – 7)	5 (4 – 7)	5 (4 – 7)	5 (4 – 7)	6 (4 – 7)	5 (4 – 7)

ACE-I: Angiotensin converting enzyme inhibitor, ARB: angiotensin receptor antagonist, IQR: interquartile range

1. Description of the other co-morbidities
2. Description of chronic lung disease (eg asthma, COPD)
3. Dependent on clinical practice

a) T-MoCA cut-off <18
b) Subjective memory or cognitive problems reported on Case Report Form and/or last 3 questions of CFS-11
c) GAD-7 cut-off 8 or more
d) CFS cut-off 4 or more

e) Significant p-values: Subjective memory impairment group CRP p=0.032; and GAD group Headache p=0.014. Significance lost when correcting for multiple comparisons.

Table 1 shows overall baseline patient clinical and laboratory characteristics from the time of enrolment in the study, including hospitalization or home care, and also stratified by neuropsychiatric outcomes. The cohort was 54% (52/97) female with a median(IQR) age of 48 (37-59) years. Self-reported ethnicities of the cohort matched the demographics of the drainage population of GSH and included: 50/97 (51%) South African Coloured, 42/97 (44%) Black African, 3/97 (3%) White South African and 2/97 (2%) Indian. The median(IQR) duration of symptoms prior to admission was 7(5-11) days and the median (IQR) PaO2/FiO2 ratio in the complete cohort was 157(74-270), indicative of the profound hypoxemia in hospitalized COVID19 infection. The commonest comorbidities included hypertension (39%) and diabetes (28%); while only four participants had HIV infection, and three participants previously had tuberculosis.

3.2 Telephonic interview timing post COVID-19 infection and neuropsychiatric outcomes

Patients in this cohort were recruited across three different COVID19 waves in SA, with 31/97 (32%), 34/97 (51%) and 32/97 (33%) from the 1st, 2nd, and 3rd waves, respectively. Our recruiting dates were June - October 2020 (Alpha), December 2020 - March 2021 (Beta) and June - August 2021 (Delta) waves respectively. Consequently, telephonic interviews were conducted a median (IQR) of 19.8 (18.5-21.1), 15.0 (14.5-16.1), and 9.2 (8.5-10.4) months post SARS-CoV-2 PCR in the 1st, 2nd and 3rd waves respectively. No participants were interviewed less than 6 months after their COVID19 infection. Figure 2 shows neuropsychiatric outcomes plotted against the time since SARS-CoV-2 PCR positivity, stratified by illness severity category. Similar numbers of poor neuropsychiatric outcomes are seen across the distribution of shorter and longer time between infection and interview, even after controlling for disease severity.

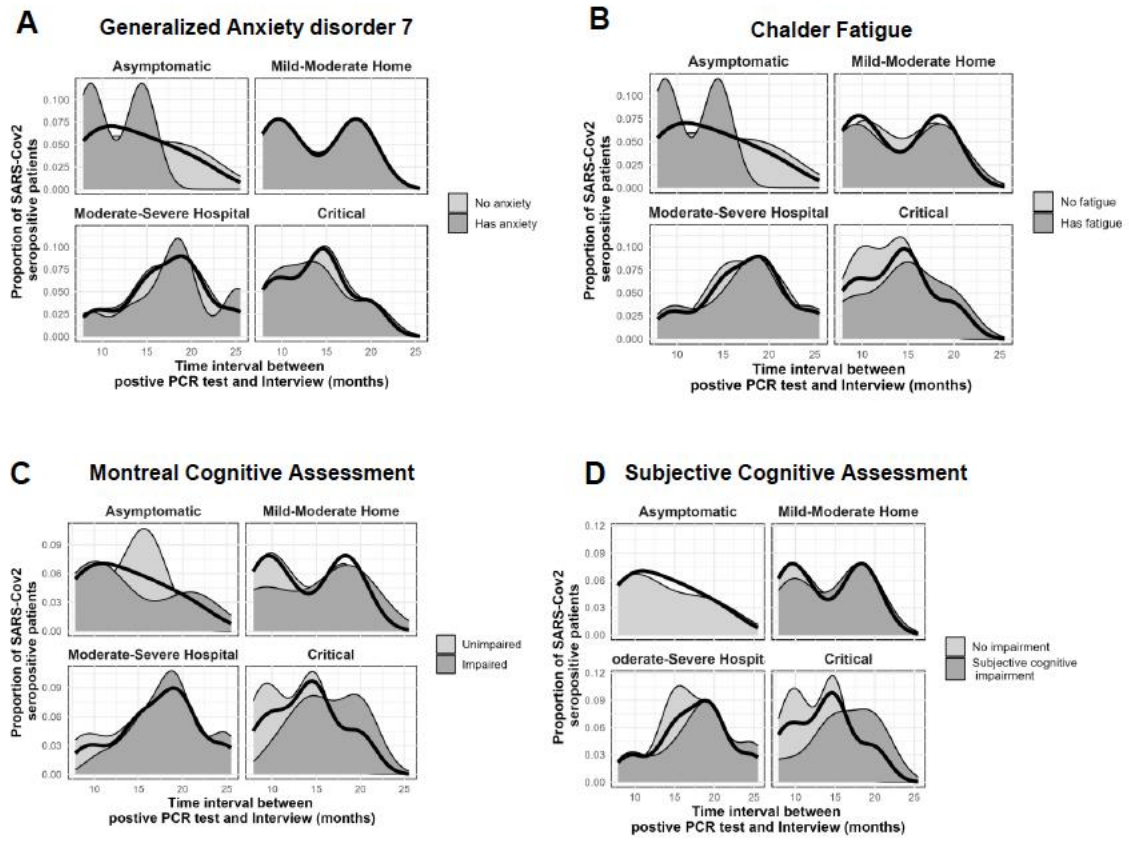


Figure 2: Neuropsychiatric outcomes by time and wave since positive SARS-CoV-2 PCR test stratified by illness severity groups

Although post-COVID interviews did not take place at a uniform time-point post-infection but rather at a range of time-points after the respective waves, one or more neuropsychiatric symptoms were consistently reported by almost half the participants regardless of duration since original infection. None of the outcomes showed a clear pattern with timing from infection/hospitalization to interview (Figure 2a-d). Furthermore, there were no significant differences identified in the distribution by wave (see Supplement **Table S4**).

3.3 Neuropsychiatric outcomes

Figure 3a-h shows neuropsychiatric outcomes in the overall cohort, stratified by illness severity. General estimates of overall rates of psychological distress, as determined with the use of the SRQ-20 (at a sensitive cut-off of 4 or more for males and 6 or more for females), found that 19/45 (42%) of males and 29/52 (56%) of females were affected at the time of telephonic interview. Overall, 25/97 (26%) of participants received mental health treatment or psychiatric medication prior to COVID19 infection, which increased to 42/97 (43%) post COVID19 infection (the original 25 and additional new 17) (Figure 3a and b); proportional increases in mental health treatment or use of psychiatric medication prior to and post infection did not differ by disease severity (p=0.15).

Using a GAD-7 cut-off of 8 or more (Figure 3c), 24/97 (25%) of participants had high anxiety levels; and hospitalized participants (moderate-severe hospitalized and critical) versus home/asymptomatic had higher anxiety levels [33% (18/53) versus 14%, (6/42) p=0.03]. Levels of fatigue measured at the various time points after participants had COVID19, as per the CFS-11 with cut-off of 4 or more (47) (Figure 3d), were very low in the asymptomatic group (23.07%) compared to the three other severity groups (59%, 64% and 59% in mild-moderate home, moderate-severe hospital and critical groups respectively, $p<0.05$ asymptomatic versus any other).

Using the T-MoCA (cut-off of ≥18 as normal), 41/93 (44%) showed cognitive impairment. The mild-moderate home severity group was least affected with only 7/29 (24%) <18, relative to 15/31 (48%) (p=0.0535) and 11/20 (55%) in moderate-severe hospital and critical disease severity groups (p =0.0269). Sixty-one-point-five percent of asymptomatic participants scored below 18 on the T-MoCA. It is important to note that only 15% of asymptomatic participants had more than 12 years of formal education compared with 42.5% across the other groups of mild, mild-moderate home, moderate-severe hospital, and critical groups combined (p=0.06). The majority of the asymptomatic participants were recruited from the BCG vaccine study, while participants from other severity categories were recruited from the ACE2 study.

Subjective memory impairment after COVID19 infection, as recorded via case report forms and/or CFS-11 memory questions, was reported by 51/97 (53%) overall, and was highest in the moderate-severe hospital group (23/33, 70%). Forty-three-point-three percent of participants

reported word finding difficulties on the CFS-11 and case report forms (Figure 3h). In many instances, participants also reported that their family members commented on this post-discharge. Further detailed descriptions of individual accounts are outlined in the online supplementary materials. Phonemic fluency, an objective T-MoCA outcome measure (Figure g), was below the 11-word normal threshold in 52/93 (56%) of participants. There were no statistical significant differences between severity groups. In all but the critical group, more participants failed than passed the phonemic fluency test, the latter in which an equal number passed or failed.

On delayed recall, 23/93 (25%) of participants had no errors, while 75% did have difficulty with delayed recall of five standardised newly learnt words on the T-MoCA. Of all participants, 15/93 (16%), 26/93 (28%), 13/93 (14%), 4/93 (4%) and 12/93 (13%) managed to retrieve 4/5, 3/5, 2/5, 1/5 and 0/5 words, respectively. Of note, all but one of the participants who lost points on delayed recall, including those who initially could not remember any words, were able to remember additional words on category or multiple choice cue.

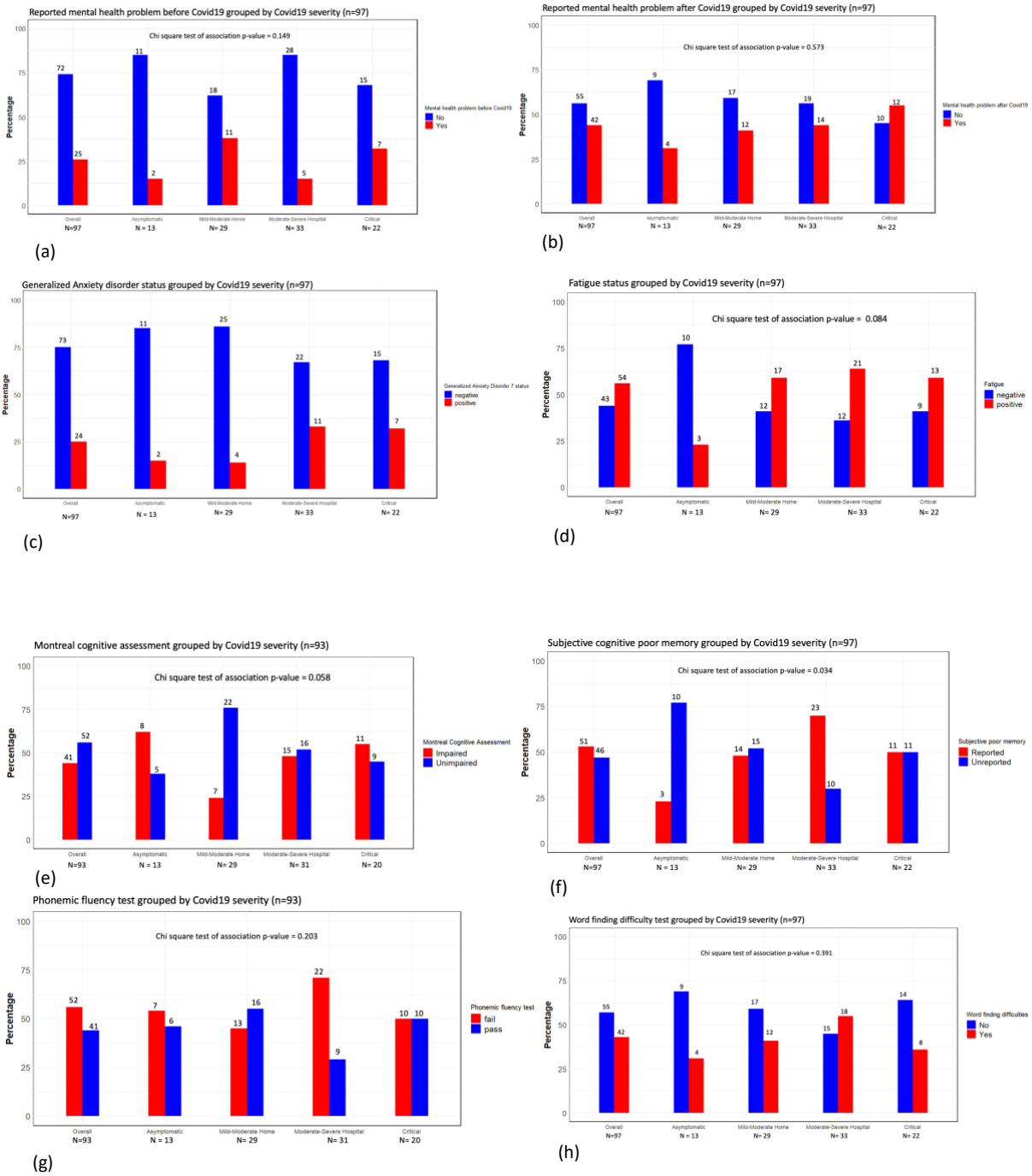


Figure 3a-h: Mental health status before and after COVID-19 and Neuropsychiatric outcomes >6 months after infection

a) Reported mental health problems prior to infection with COVID19 overall (far left) and in each different COVID19 severity group

b) Reported mental health problems after infection with COVID19 overall (far left) and in each different severity group

c) Proportion of patients screening positive for significant anxiety post-COVID infection overall (far left) and in each severity group

d) Proportion of patients screening positive for significant fatigue post-COVID infection overall (far left) and in each severity group
e) Objective impaired vs unimpaired overall T-MoCA results post-COVID infection as recorded for participants overall (far left) and in each severity group
f) Subjective impaired vs unimpaired memory post-COVID infection as reported by participants overall (far left) and in each severity group
g) Objective impaired vs unimpaired phonemic fluency ability as recorded on the T-MoCA for participants post-COVID infection overall and in each severity group
h) Subjective word finding difficulties as reported by participants post-COVID infection overall and in each severity group

3.4 Inflammatory, cardiovascular and RAS and neuropsychiatric outcomes

Peak illness severity and recovery blood O-link analyses were performed on samples from 77% (75/97) and 75% (73/97) of interviewed participants, respectively. Peak illness severity was determined after longitudinal sampling was performed, and clinical data was collected at each sampling time-point. This data allowed classification of illness severity. The peak illness severity was therefore determined as being the time-point of maximal symptoms/laboratory derangement. A table in the online supplementary materials shows sampling stratified by neuropsychiatric outcome and disease severity groups (see **Table S5**).

Using either peak (Figure 4a to d) or recovery (see Supplementary materials **Figure S7B**) samples, neither principal component analyses of proteins using normalized protein expression (NPX) levels or unsupervised hierarchal cluster analyses could differentiate between affected and unaffected individuals for any of the persistent neurocognitive outcomes; in contrast to the clear pattern of clustering associated with different disease severity groups. The analyses presented in the Heatmaps and Principle Component Analysis plots in Figure 4 and the Supplementary materials include biomarkers of RAS, antibody responses, inflammatory and cardiovascular disease; similar analyses were conducted by limiting proteomic data to one panel e.g. inflammatory or cardiovascular, and with and without antibody and/or RAS data, but the results were similar.

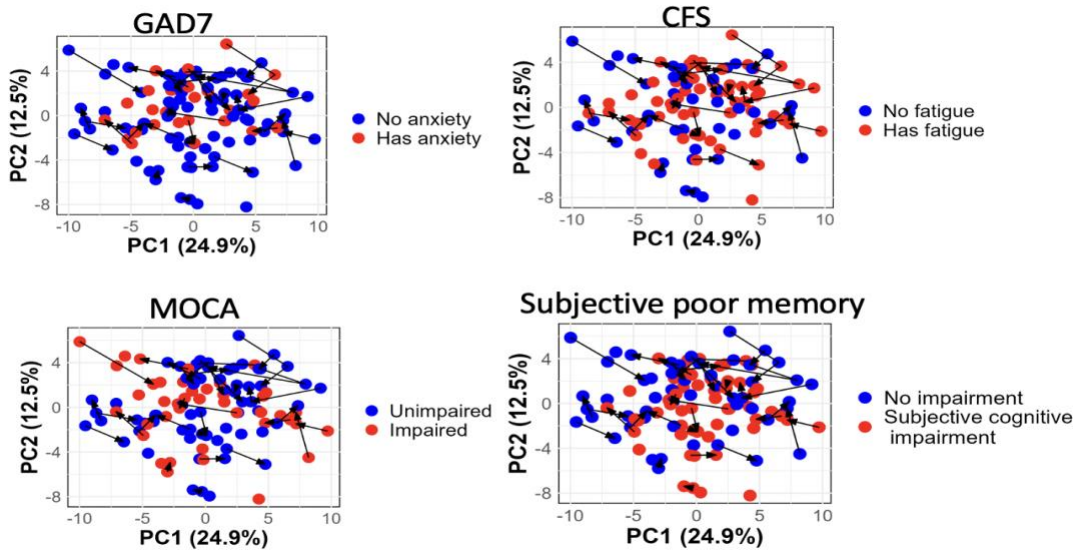


Figure 4a: O-link inflammatory panel PCA plot

a) PCA plot depicting O-link inflammatory panel results at peak illness severity for participants with (red) or without (blue) anxiety post-COVID infection

b) PCA plot depicting O-link inflammatory panel results at peak illness severity for participants with (red) or without (blue) fatigue post-COVID infection

c) PCA plot depicting O-link inflammatory panel results at peak illness severity for participants with or without impaired total T-MoCA scores post-COVID infection

d) PCA plot depicting O-link inflammatory panel results at peak illness severity for participants with or without subjectively impaired memory post-COVID infection

Please see Supplementary materials, **Table S2**, for a list of markers included in our proteomic analysis. When studying these Heatmaps carefully, overlapping markers identified by other researchers such as Il-6, TNF and CCL11 (9, 10) were strongly presented but not at the level of statistical significance. Other individual markers highlighted by researchers, namely NFL, and CRP, were unfortunately not included in our O-link panels. CCL-11 or eosinophil chemotactic protein (eotaxin) was a marker of interest since it has been strongly implicated as an immune marker elevated in a variety of ageing, neurodegenerative, neuropsychiatric, and neuroinflammatory conditions (35). Eotaxin can be rapidly transported through the BBB, it is associated with allergic conditions, it has biphasic effects as either neuroprotective or neurotoxic, it may downregulate BBB junction proteins, it is released by activated astrocytes, causing oxidative stress and microglial activation, leading to neurotoxicity (35). Furthermore, it has been strongly associated with the hippocampus (35), which could explain memory deficits. However there were no statistically significant elevations in CCL-11 seen in our cohort differentiating those participants who expressed persistent symptoms from those who did not.

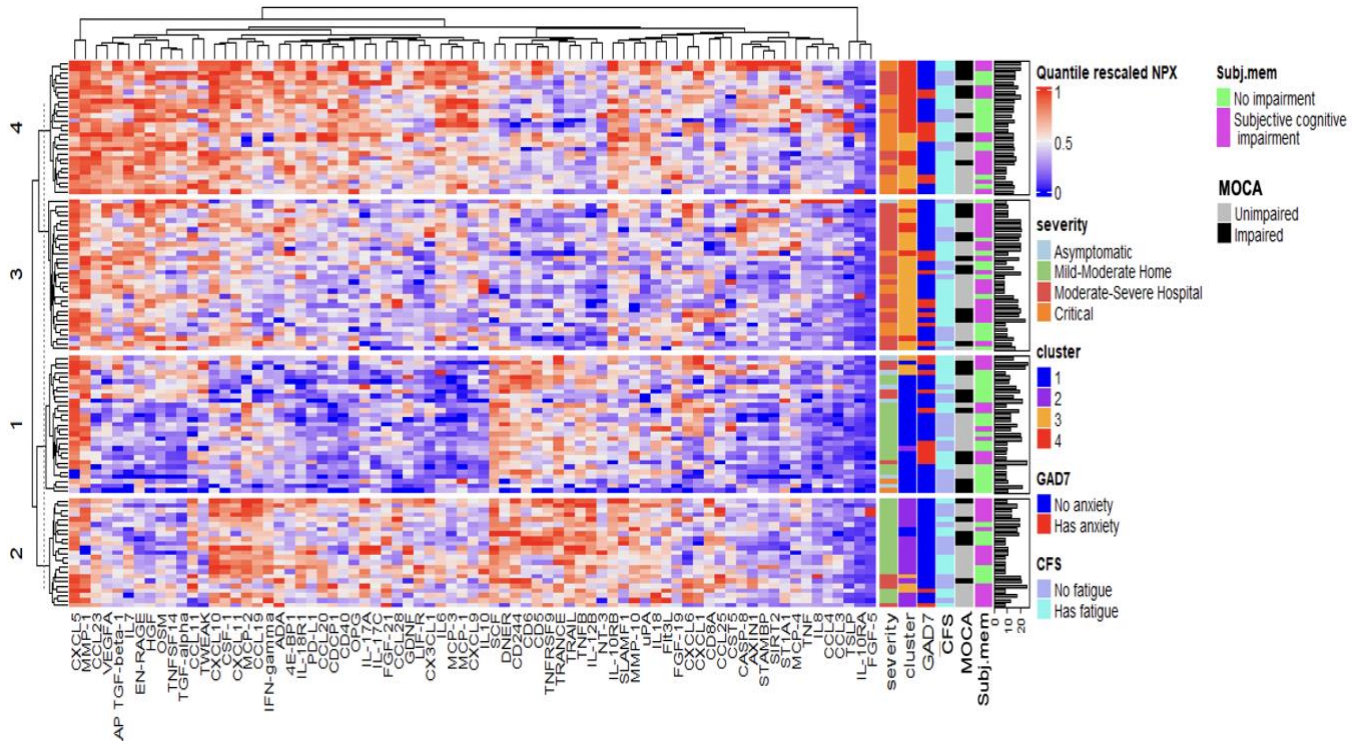


Figure 4b: O-link inflammation Heatmap at Peak

* Rows represent study participants, showing expression of different proteins in each participant

** Each protein and analyte are represented in different columns

*** On the far right, columns represent severity groups

Figure 5a-d shows RAS markers for anxiety, fatigue, T-MoCA and subjective memory impairment. We explored ACE and ACE2 activity and a reciprocal relation to disease severity was discovered between these two analytes. Comparing those with and without persistent neuropsychiatric deficits at each severity category, no RAS marker (either at peak or recovery) differed amongst affected and unaffected individuals for any of the neuropsychiatric outcomes.

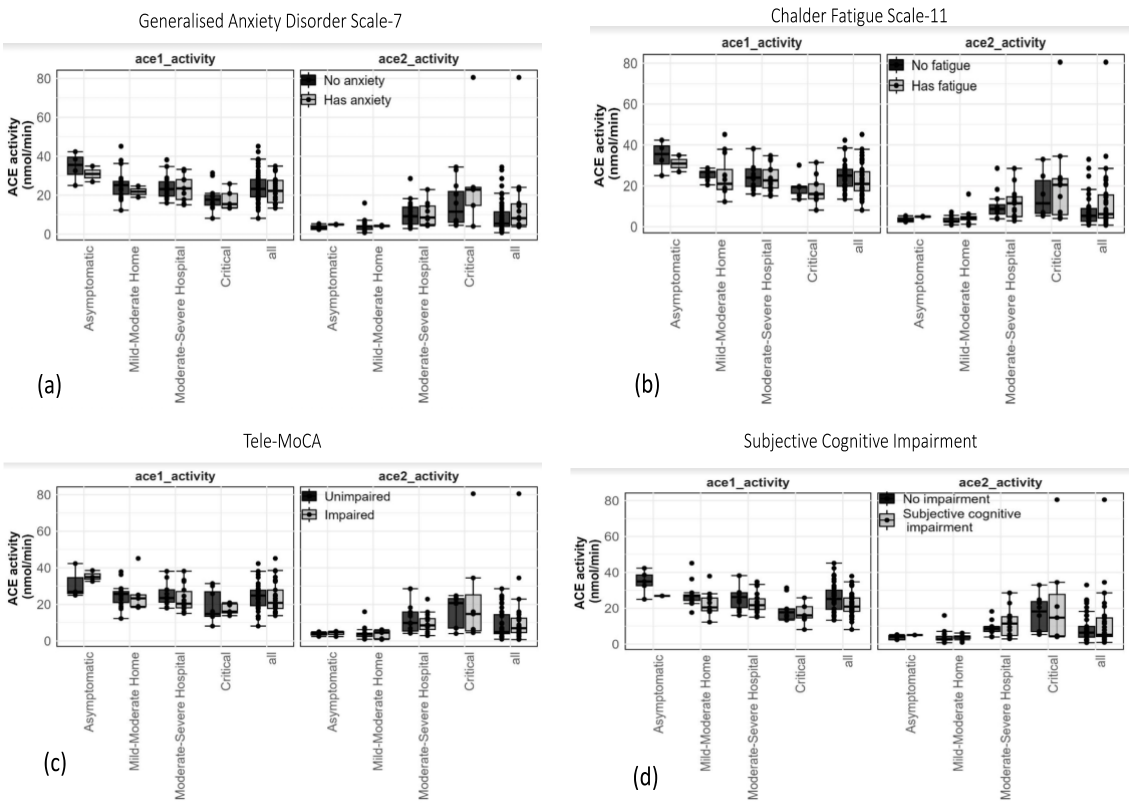


Figure 5a-d: ACE and ACE-2 activity across severity groups in different neuropsychiatric outcomes

a) ACE1 and ACE2 results in participants with (light) or without (dark) anxiety post-COVID infection overall (far right) and for the respective severity groups

b) ACE1 and ACE2 results in participants with (light) or without (dark) fatigue post-COVID infection overall (far right) and for the respective severity groups

c) ACE1 and ACE2 results in participants with (light) or without (dark) impaired total T-MoCA scores post-COVID infection overall and for the respective severity groups

d) ACE1 and ACE2 results in participants with (light) or without (dark) subjectively impaired memory post-COVID infection overall and for the respective severity groups

Figure 5e-h depicts adaptive immune response to SARS-CoV-2 in the different categories of severity as measured by anti-nucleocapsid and anti-spike IgG response titres. By looking at anti-nucleocapsid IgG response, we looked at natural immunity. Anti-spike IgG response can be affected by vaccine-induced immunity, while anti-nucleocapsid IgG response can only be induced through natural infection. Overall, as disease severity increased, antibody responses increased, yet no significant differences existed between those participants who developed neuropsychiatric sequelae in each severity category vs those who did not.

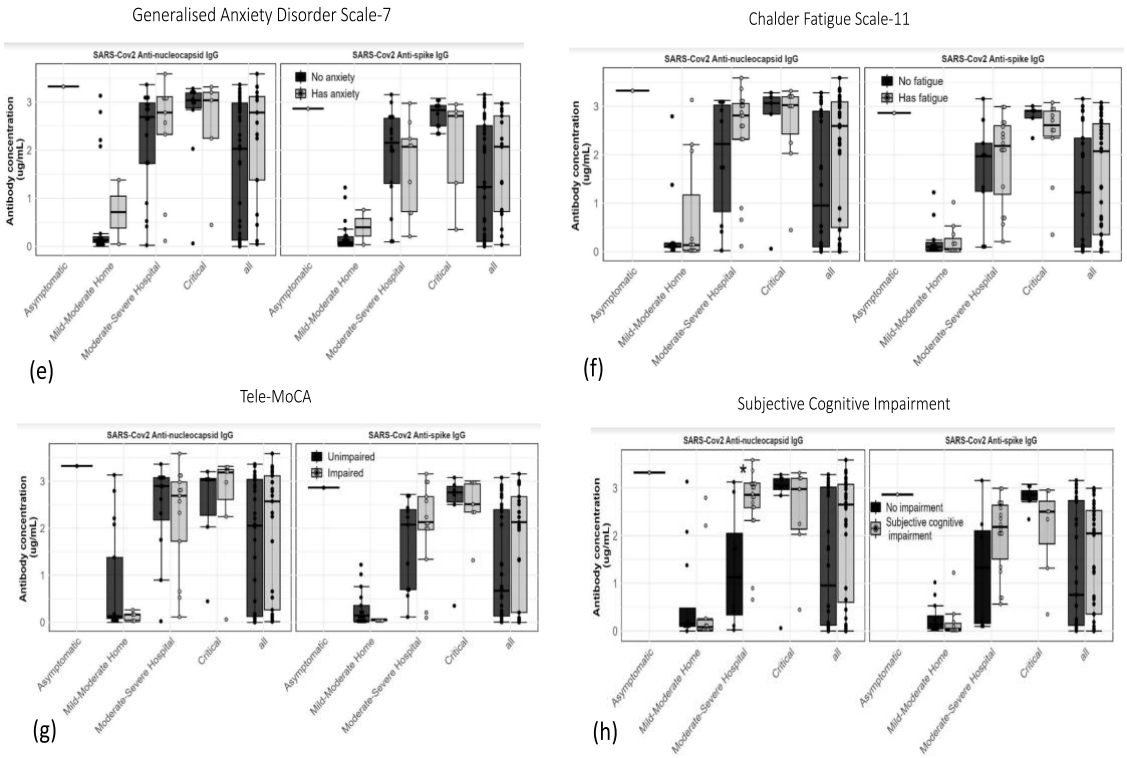


Figure 5e-h: Antibody responses to SARS-CoV-2

e) SARS CoV2 anti-nucleocapsid and anti-spike IgG antibody responses in participants with (light) or without (dark) anxiety post-COVID infection overall (far right) and for the respective severity groups

f) SARS CoV2 anti-nucleocapsid and anti-spike IgG antibody responses in participants with (light) or without (dark) fatigue post-COVID infection overall (far right) and for the respective severity groups

g) SARS CoV2 anti-nucleocapsid and anti-spike IgG antibody responses in participants with (light) or without (dark) impaired total T-MoCA scores post-COVID infection overall and for the respective severity groups

h) SARS CoV2 anti-nucleocapsid and anti-spike IgG antibody responses in participants with (light) or without (dark) subjectively impaired memory post-COVID infection overall and for the respective severity groups

* Asterix indicates significant p-value

4. Discussion

Our study examined longer-term neuropsychiatric sequelae (median of 19.8, 15.0, and 9.2 months post SARS-CoV-2 PCR in the 1st, 2nd and 3rd waves respectively) in a young South African cohort with very low rates of pre-morbid psychiatric illness or cognitive impairment. We explored whether the RAS and 96-plasma inflammatory markers measured during peak illness severity or a recovery time-point (>3 months from COVID19) were associated with neuropsychiatric symptoms. Despite the long duration from acute COVID19 illness, more than half of all participants were experiencing persistent neuropsychiatric sequelae, including: 49%, 25% and 55% of participants with psychological distress (SRQ-20), anxiety (GAD-7: 8 or more), and fatigue (CFS-11: 4 or more) respectively. Ongoing subjective memory and cognitive problems were reported in more than half of participants, while slightly less showed evidence of objective cognitive and/or memory impairment on the T-MoCA. Of these outcomes, only persistent fatigue showed statistically significant association with peak illness severity. There were no differences in ACE activity linked to the presence or absence of neuropsychiatric sequelae. In fact, neither abnormal RAS physiology, specific plasma inflammatory signature, nor antibody response at either peak illness or recovery time-points was significantly associated with any persistent neuropsychiatric or cognitive sequelae (please see Supplementary materials **Figure S6B and S7B** for additional graphs).

The high rates of persistent psychiatric and cognitive sequelae in this young South African cohort are noteworthy. This is consistent with several studies in other contexts. Pooled prevalence rates of neuropsychiatric symptoms post-COVID in a large recent systematic review including 1, 285, 407 participants from thirty-two countries were 20% at 3-6 months, and 18% at six or more months (36). More specifically, prevalence rates of persistent symptoms among survivors recovered from acute illness were 28.7% for fatigue (in their study categorised under generalised symptoms); 18.3% for depression; 16.2% for anxiety; 19.7% for cognitive deficits; and 17.5% for memory impairment (36). Albeit not reaching the level of statistical significance, the latter review found a declining trend for both neurological and psychiatric symptoms as compared across the <3 months, 3-6 months, and 6-12 month time-points (36). A large South African study including 4685 recruited people from across the country, 3700 of whom completed 6-month assessments, found that just under half of hospitalized and non-hospitalized patients reported one or more persistent symptoms, including neuropsychiatric, at

6-months (14). This study investigated outcomes across Beta, Delta and Omicron waves: 11.7% of non-hospitalized patients and 32.1% of hospitalized patients experienced persistent fatigue, and 10% of hospitalized patients experienced lack of concentration at 6-month follow-up (14). In a different South African cohort, rates of persistent fatigue was found to be 50.3%, and confusion or lack of concentration 17.5%, at the 3-month mark (13). Similar to our findings, persistence of fatigue has been linked to severity of acute illness (37). Persistent fatigue has been linked to other severe illness groups requiring prolonged intensive care admission and ventilation as well as other viral infections (37, 38). Of all the psychiatric outcomes, we noted persistent anxiety rates to be lowest. This is consistent with a two-year retrospective cohort study of more than a million patients in the USA that found anxiety and mood disorders post-COVID to be transient, while the increased risk of cognitive deficit, dementia, psychotic disorder, and epilepsy or seizures persisted throughout the lengthy follow-up period (7, 37). We postulate that over time, anxiety symptoms in our cohort may have decreased or resolved while cognitive symptoms persisted.

The high rates of self-reported memory and cognitive impairment (which were confirmed on objective testing on the T-MoCA) are consistent with a range of work. In the COVCOG study, researchers predicted that participants who had self-reported cognitive symptoms will reflect as such in objective tests (24). With the exception of the asymptomatic group, this was true for our cohort given that both objective and subjective cognitive impairment had similar prevalence in the remainder of the cohort (mild, moderate-severe, severe, and critical groups). The COVCOG study particularly found reduced memory performance on multiple verbal memory tasks (24), while Guo et al reported that cognitive dysfunction affected 70% of post-COVID patients in their mixed cross-sectional/longitudinal online study population, with forgetfulness, word-finding problems, and semantic dysfluency being the commonest deficits (39). We noted a similar pattern with the commonest deficits being for delayed recall, phonemic fluency, and self-reported word-finding difficulties. Our study did not measure semantic dysfluency as it is not included in the T-MoCA. Of note that while high rates of impairment were found on delayed recall, all but one of the participants were able to recall additional words on category or multiple choice cueing. This suggests that while encoding remains relatively intact, the deficit lies with retrieval of learnt information, called ‘dysexecutive amnesia’ (23), clinical features consistent with subcortical white matter damage (40). Microglial activation and microinfarcts are key pathophysiological mechanisms responsible for COVID19-related white matter damage (41). Objective cognitive impairment

was prevalent across the spectrum of disease severity. Similar to other studies, impairment on objective testing was found in more than a third of participants with mild disease (24, 42); and has been reported to be nearly 70% in a USA neurology clinic study amongst survivors with mild to moderate COVID19 disease severity (43). The mean age of our cohort was under 50 years, and only a single participant reported pre-existing memory changes. Thus, the new onset of these cognitive symptoms within two years following COVID19 infection suggests that these are attributable to COVID19 infection.

The cytokine 'storm' associated with acute respiratory distress and severe COVID19 has been a much studied hallmark of the disease and anti-inflammatory treatments remain a cornerstone of management (10, 44, 45). It is postulated that the virus-induced cytokine storm could initiate a central inflammatory response, setting in motion a cascade of events by reducing serotonin production which in turn could attenuate downstream monoamine neurotransmission, increase brain glutamate excitotoxity, and upregulate N-methyl-D-aspartate (NMDA) receptors (15). This neurotransmitter-driven excitotoxicity, as well as microglial activation (10), can be linked to synaptic pruning and neuronal loss (15). However, despite examining serum samples taken at both peak illness severity and a recovery time-point more than three months post infection, we did not see a correlation with any of the persistent neuropsychiatric sequelae. Several possible explanations may account for this finding. First, the intensity, duration, and resolution of neuroinflammatory processes such as microglial activation may be distinct from systemic inflammation, and consequently plasma/serum rather than CSF sampling during acute illness may be uninformative. Second, the determining pathophysiological mechanisms may be non-immune e.g. direct neuronal injury by SARS-CoV-2, or through viral persistence (10, 44). Thirdly, the mechanism of immune-related neuronal injury may either not be adequately represented by 96-inflammatory biomarkers selected such as that a separate neurological protein signature for COVID19 severity may exist (46). If such mechanism entails autoantibody damage as some have argued (10, 46), it may not be reflected by plasma/serum inflammatory markers. Fourthly, and perhaps most importantly, psychosocial factors could have influenced the results. While our study focuses primarily on biological causal variables, we acknowledge that the biopsychosocial impact of the COVID19 pandemic should not be underestimated, and not neglected in humanitarian efforts to assist those affected (47, 48). Indeed, psychosocial consequences on study participants in South Africa could possibly explain why there was no clear association found between biomarkers and eventual neuropsychiatric outcomes.

Despite incomplete understanding of pathophysiological mechanisms underlying neuropsychiatric symptoms of long COVID, both pharmacological and non-pharmacological treatments have shown effect and are now being recommended (15, 49). The latest collaborative consensus guidance statement on the assessment and treatment of mental health symptoms in patients with post-acute sequelae of SARS-CoV-2 recommends serotonin-norepinephrine reuptake inhibitors (SNRI's) for the treatment of dysautonomia by increasing cardiac sympathetic control and decreasing vagal activity (15). While selective serotonin reuptake inhibitors (SSRI's) can cause peripheral vasoconstriction and decrease pooling (15), they can help by inhibiting mast cell activation, and their effects on platelets can decrease micro-clotting in the setting of COVID infection (15). Another antidepressant agent, bupropion, may be used to improve cognitive dysfunction; anxiety disorders can be treated with gabapentin, which has also shown efficacy in treatment of neuropathic pain (15). At times, benzodiazepines may be selected to relieve acute anxiety, however they are not recommended in patients with post-traumatic stress disorder (PTSD) as they impair anterograde memory (15). Also with regards to PTSD, the alpha-adrenergic antagonist prazosin has shown promise reducing nightmares (15). The third-generation antipsychotic aripiprazole has shown benefit in the treatment of fatigue related to myalgic encephalitis / chronic fatigue syndrome (15), both conditions which are linked to various viral aetiologies not limited to COVID only.

Non-pharmacological treatments should start with forming a strong therapeutic alliance and validation of the patient's concerns (15). A biopsychosocial(cultural-spiritual) model is employed (15). Maintaining patient autonomy and control over choices is important; and various forms of psychotherapy, such as cognitive behavioural therapy (CBT), supportive psychotherapy, group psychotherapy and self-help resources are recommended (15). Cognitive concerns should be addressed as early as possible (49), especially where these may halt a neurodegenerative process or may facilitate earlier return to prior levels of functioning. Principles from the consensus guidance statement include energy reservation, especially for patients with fatigue, and graded return to normal activity (49). Patients are advised to decrease screen time, manage stress, incorporate sleep hygiene, and increase physical activity. Cognitive rehabilitation techniques include cognitive strategies, cognitive remediation, and compensatory techniques (49). The goal is to resume exercise, and return to work as well as avocational interests (49). It is important not to neglect the treatment of underlying mental and physical conditions, and attempt to minimize polypharmacy (15). Throughout the process, collaborative

multidisciplinary management is advised as well as close monitoring of return to normal activities so as not to exhaust patients with already depleted reserves (49).

The aetiology of psychiatric illnesses is often multifactorial and researchers are now emphasizing the biopsychosocial(cultural-spiritual) approach to the management of long COVID (47). Thurner and Stengel are calling for managing patients by treating known diseases on a biological level; incorporating physiotherapy and other rehabilitation methods; screening for and treating psychological conditions, especially aiming to reduce avoidance behaviour and decrease anxiety; neurocognitive training is advised; and the role of Occupational Therapists emphasised to train and/or rehabilitate necessary abilities and enable work-life participation for affected patients (47). It is acknowledged that some "physical" symptoms may be due to somatization, which in itself cause reduced ability to express affects/emotions. Healthcare professionals are advised to be open to recognizing, validating and discussing these affects (47). On the local front, the global economic crisis caused by COVID19 could have affected poorer communities in South Africa perhaps more than richer communities; lockdowns had a major impact on domestic matters and earning a living for ordinary South Africans; restrictions and wearing masks could have diminished the ability of people to read facial cues and appropriately respond to each other while being in lock-down. This in a country with already high rates of intimate partner violence which may profoundly affect mental health (48).

5 Limitations

The study has a few limitations. First, our sample size is small given the time from infection/admission to interview contacts; fortunately our sensitivity analysis supports the absence of selection bias. Reduced sample size may have limited our ability to see small but significant differences, and also impact the power to detect differences across the waves. Second, knowing the confounding effects of education on cognitive testing, educational levels and not only COVID19 infection could have affected the Tele-MoCA results in this group (22, 40, 50). Our asymptomatic group arose from two different cohorts with differing background education levels; this may account for the higher than expected rates of "objective" cognitive impairment in this group as fewer years of formal education could place those participants at a disadvantage, resulting in lower overall T-MoCA scores unrelated to COVID. Related to this,

we did not include other control groups such as a hospitalized age-matched group of patients with a different illness, meaning that some of these findings may not be specific for SARS-CoV-2 infection. Third, while change in neuropsychiatric symptoms from pre-COVID cannot be assessed objectively, the availability of subjective reports on pre-COVID mental health status was obtained from participants. The retrospective nature of reports of pre-COVID mental health symptoms and treatment, as well as the fact that the reports have all been done at one time-point – participants were asked to reflect back on mental health symptoms and report in the present time about neuropsychiatric symptoms experienced, both reports done at the time of this once-off post-COVID telephonic interview – could have introduced bias. Fourthly, we focused on peripheral inflammatory markers and the RAS in plasma only, and our O-link analysis did not include specific neuronal markers or examine the CSF. Fifthly, and perhaps most importantly, we did not address psychosocial stress experienced by participants per se.

6 Conclusion

In summary, our findings confirm a high and prolonged impact of acute COVID19 infection on a cohort of relatively young South Africans throughout the first three COVID waves. These findings are consistent with other findings in HIC, and are of concern from both an individual as well as public health perspective. With the large numbers of people affected, even a proportion who may not be functioning at previously attained levels can have a ripple-effect on the broader economy. Clinicians should be alerted to the possibility that persistent cognitive and memory problems as long as two years or more following infection with SARS-CoV-2 can occur in patients across the disease severity spectrum, with common problems being fatigue and dysexecutive amnesia. All patients reporting new or exacerbated neuropsychiatric symptoms persisting for greater than six months after COVID19 infection deserve that their complaints be taken seriously by treating clinicians; that thorough history-taking and examination be performed which could include screening questionnaires such as those used in this study; and that treatment and support for anxiety, fatigue, memory, and cognitive problems be offered.

In contrast to other consequences of infection, long-term neuropsychiatric sequelae may have a complex pathophysiology that is not easily linked to systemic inflammation either at acute or

recovery time-points, meaning simple plasma biomarkers capable of risk-stratification for increased support or other treatments may not be possible. However, it may be precisely in this realm that the psychosocial effects of COVID19 may influence symptom expression. In fact, new hope for psychophysiological treatments which have shown efficacy, as found by other research teams (15, 47, 49), strengthen the relevance of our psycho-neuro-immunological data presented in this paper. This research could possibly help to improve the general conceptualization of these complex psychopathological disease processes.

Future research could focus on sampling all compartments at the same time, namely conducting serum as well as CSF sampling in order to investigate and compare inflammation in the brain with systemic inflammation at set time-points; additionally, looking at neutralizing antibody effectiveness and responses over the different COVID19 waves could provide answers on the adaptive immune response front; lastly, but not least, looking at psychosocial variables and contributors in more depth may provide important insights on why some patients develop neuropsychiatric sequelae, and why some do not.

Online Supplementary Materials

The following are the Supplementary data to this article
Supplement to:
Van Niekerk I, Panieri M, Müller T, Mapahla L, Day C, Dzanibe S, Stein DJ, Peter JG. Persistent >6 months Neuropsychiatric symptoms in a cohort of SARS-CoV-2 PCR positive patients in Cape Town, South Africa

Supplementary Methods: Study setting and severity classifications
GSH description

Groote Schuur Hospital (GSH) opened in 1938 and has a bed capacity of 893. The hospital serves the entirety of the Western Metropol (41). The hospital offers both tertiary-level services, receiving referrals from three secondary-level hospitals (Victoria Wynberg Hospital, New Somerset Hospital, and Mitchell's Plain District Hospital) as well as secondary-level services. The patient population has high rates of communicable and non-communicable diseases. Attending clinical teams consist of consultant physician led teams in various specialties, usually consisting of medical interns, medical officers, and specialist registrars (trainees) (51). Sub-specialist patient consultation is readily available. Full radiology and laboratory services are available 24 hours a day. Normally, the Critical Care Division at GSH maintains 25 Intensive Care Unit beds with a nurse to patient ratio of 1:2 (51). These services span across medicine, surgery, cardiothoracic surgery, and neurosurgery (51).

During the COVID-19 pandemic the ICU beds were more than doubled from 25 to 54 beds (51) with additional non-COVID ICU capacity to provide mechanical ventilation. In addition the two existing high care units (HCU) and a general ward were converted to high flow nasal oxygen (HFNO) units providing 44 beds. The major limiting factor in these expansions was the availability of nursing staff, while doctors from all departments were divided into a rotational COVID response team to reduce burnout and ensure that non-COVID services were still functional. Over a 13 month period 461 patients were admitted to ICU for mechanical ventilation (51).

Due to significant resource constraints (despite the increase in existing ICU and HCU resources) the Critical Care team in the Western Cape developed an objective COVID-19 triage tool to ensure equity and that the patients with the best predicted outcomes were ensured access to mechanical ventilation or HFNO (http://www.sajcc.org.za/public/sup/503.pdf).

This triage system was scored based on:

1. An acute severity score using the Sequential Organ Failure Assessment (SOFA) score. The SOFA is based on the PaO2, fraction of inspired oxygen, mechanical ventilation status, the Glasco coma scale, Mean arterial pressure, platelet value, creatinine value, and total bilirubin.(52, 53)

2. Baseline functional status: using the clinical frailty score (54), and/or the Eastern Cooperative Oncology Group (ECOG) performance status (55)
3. Comorbidities scoring: assessed the 5-10 year mortality risk based on the number and severity of comorbid illness

Each section was allocated a numerical score, and the scores were then summed, ranging from 1-15 points. This triage tool divided patients into four categories based on the point scores:

- Red: 1-3 points: highest priority for ventilatory support
- Orange: 4- 6 points: intermediate priority for ventilatory support
- Yellow: 7-9 points: low priority for ventilatory support
- Green: 10-15 points: lowest priority for ventilatory support

COVID-19 infection definition and severity classification

COVID-19 infection was defined predominantly as requiring a laboratory confirmation (predominantly PCR-positivity, although in 2 cases SARS-CoV-2 antigen positivity was also accepted) irrespective of clinical signs symptoms (56, 57), alternatively by highly suggestive clinical criteria as per WHO Probable Case definition (1 case only).

COVID-19 illness severity was determined at all sampling timepoints and at hospital admission or COVID-19 testing (for participants not hospitalized), as the earliest point when clinical data were available – irrespective of whether it was possible to sample at this point. The most severe category (including at admission/testing) was then taken as the overall severity for each participant. The severity scoring system **(Table S1A)** was based firstly on the WHO's COVID-19 disease severity classification (18). The only modification was to evaluate the ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) according to the National Institutes of Health (NIH) and US Food and Drug Administration (FDA) guidelines (20, 58). Thus, participants not meeting other criteria for critical disease (e.g. no organ failure, shock, or high flow nasal oxygen (HFNO) or mechanical ventilation (MV) use) but with either mild and moderate acute respiratory distress syndrome (58) with a PaO2/FiO2 ratio of $\leq$300 but >100, were considered to have severe COVID-19, rather than critical COVID-19, as would have been the case following only the WHO guidelines.

It proved essential to distinguish between ambulatory and hospitalized participants in analyses, and thus COVID-19 severity was further defined according to the National Institute of Allergy and Infectious Diseases (NIAID) 8-point ordinal scale (59) (**Table S12**), and subsequently stratified into four groups: Asymptomatic PCR-proven COVID-19 infected; NIAID 1-2 (ambulatory, WHO mild-moderate disease); NIAID 3-5 (hospitalized, WHO moderate-severe disease, including PaO2/FiO2 $\leq$ 300 and > 100); NIAID 6-7 (hospitalized with WHO critical disease or PaO2/FiO2 $\leq$ 100). Moreover, the peak severity timepoint for each participant was identified by 1) the worst COVID-19 severity which occurred, 2) where multiple timepoints had the same maximum COVID-19 severity score, by clinical criteria based on vitals and overall clinical progression, in which respiratory rate, oxygen saturation (based on pulse oximetry) and symptoms reported played key roles.

Categories are defined by any (not necessarily all) of the criteria being present, and for critical disease this applies in a cumulative fashion – where criteria must be met for Severe disease, with additional findings as per the Critical criteria above. Shock was defined as a blood pressure reading of <90/60 mmHg. N/A – not applicable, i.e criterion types which were not determining

factors at a particular severity; PaO2/FiO2 - the ratio of arterial partial pressure of oxygen to fraction of inspired oxygen; SpO2 - oxygen saturation measured by pulse oximetry.

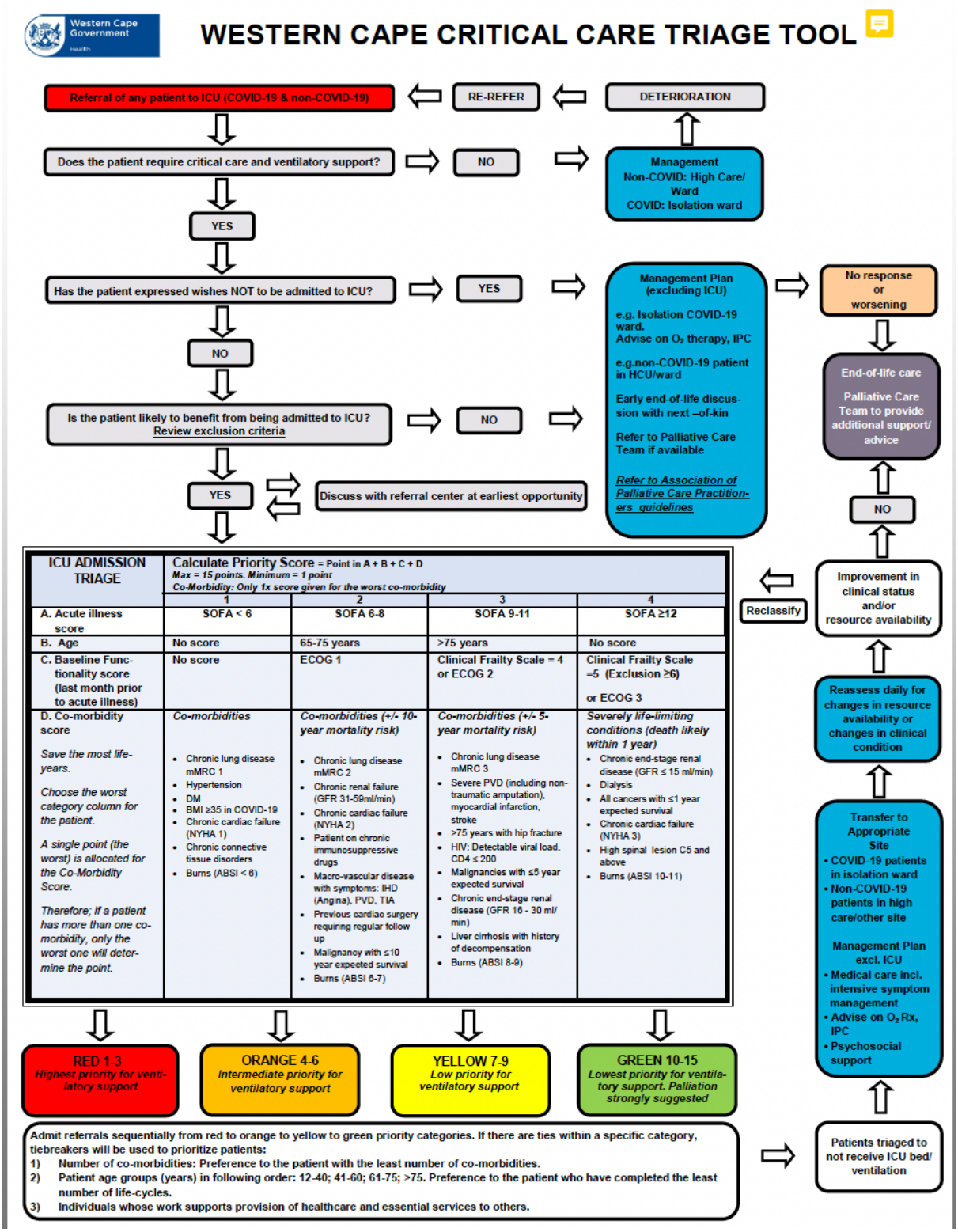
Table S1A: WHO Severity Classification

COVID-19 Severity	Symptoms	Respiratory Rate	SpO2 on room air	Supportive oxygen therapy	PaO2/ FiO2	Complications
Asymptomatic	None	N/A	N/A	None	N/A	N/A
Mild	Present	<20	N/A	None	N/A	No signs of viral pneumonia or hypoxia
Moderate	Present	≥20	≥90%	Nasal prongs or Face Mask (40% /non-rebreather)	>300	None - not severe pneumonia
Severe	Present	≥30	<90%	Nasal prongs or Face Mask (40% /non-rebreather)	>100 & ≤ 300	Severe respiratory distress
Critical	Present	N/A	N/A	High flow nasal oxygen or mechanical ventilation	≤ 100	Sepsis, shock, multi-organ dysfunction/failure

Table S1B: National Institute of Allergy and Infectious Diseases (NIAID) Severity Classification

NIAID Score	Definition
1	Not hospitalized and no limitations of activities
2	Not hospitalized, with limitation of activities, home oxygen requirement, or both
3	Hospitalized, not requiring supplemental oxygen and no ongoing medical care
4	Hospitalized, not requiring supplemental oxygen but with ongoing medical care
5	Hospitalized, requiring supplemental oxygen
6	Hospitalized, requiring non-invasive ventilation or use of high-flow oxygen devices
7	Hospitalized, receiving invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO)
8	Death

Western Cape Critical Care Triage Tool



WESTERN CAPE CRITICAL CARE TRIAGE TOOL

IN-ICU DECISION TOOL:

Re-assess all patients admitted to ICU at 48 hours and at 120 hours after admission.

Reclassify the PRIORITY CATEGORY using; 1) Baseline SOFA score at admission, 2) SOFA after 48 hours, 3) SOFA after 120 hours.

Principles of re-assessment:

1) Once a patient has been accepted into the ICU (guided by the PRIORITY CALCULATOR FOR ADMISSION above), progression IN-ICU is now monitored by using the initial SOFA score as baseline in relation to the follow-up SOFA scores (delta-SOFA). The SOFA score can STAY THE SAME (not ideal), IMPROVE (desired) or DETERIORATE (worst).
2) After re-assessment (at 48 hours and 120 hours after admission) the patient can only stay in the SAME PRIORITY CATEGORY if the SOFA has IMPROVED.
3) If the SOFA score stays THE SAME in a re-assessment, the patient must move to the next LOWER PRIORITY CATEGORY
4) If the SOFA IMPROVES, the patient can either stay in the SAME PRIORITY CATEGORY, or move into a HIGHER PRIORITY CATEGORY, depending on the amount of SOFA score improvement.

SOFA < 6	SOFA 6-8	SOFA 9-11	SOFA 12 and more
Highest priority for continued ICU support	*Intermediate priority for continued ICU support*	*Low priority for continued ICU support*	*Lowest priority for continued ICU support*

Continue with critical care and re-evaluate and reclassify at 48 hours after ICU admission, 120 hours after ICU admission and then every 24 hours thereafter

When the patient moves into a lower priority after reassessment, the highest category patient will get preference. If there is no competition for resources, care will continue as is.

It is highly recommended that a decision-making team or SPECIALIST ICU UNIT help facilitate this process.

End-of-life care

Withdrawal of ventilatory support as per guidelines

Palliative Care Team to provide additional support/advice

Exclusion criteria for admission to ICU

- Patient expressed wish not to be admitted to ICU / advance directive
- Clinical Frailty Scale ≥6 and more
- ECOG score 4 (Eastern Cooperative Oncology Group)
- < 6 months life-expectancy
- Unwitnessed cardiac arrest
- Severe and irreversible neurological injury (GCS<6: motor score <4)
- Irreversible age-specific hypotension unresponsive to fluid resuscitation and vasopressor therapy
- Severe baseline cognitive impairment (inability to perform ADL)
- Chronic respiratory disease with poor functional capacity – mMRC 4
- Cardiovascular disease - NYHA 4 or known poor ejection fraction on maximal medical therapy
- HIV/AIDS with an AIDS defining illness
- CD4 ≤100 and/or VL ≥10 000 c/ml
- Severe burns with high predicted mortality (ABSI ≥ 12)
- Liver cirrhosis - Child Pugh ≥7 or MELD ≥20
- Advanced untreatable neuromuscular disease
- Chronic kidney failure in patient not eligible for dialysis
- End stage organ failure and not a candidate for transplantation

Sequential (Sepsis Related) Organ Failure Assessment

Score	0	1	2	3	4
Respiratory					
PaO_2/FiO_2, mmHg	Normal	<400 (53.3)	<300 (<40)	<200 (26.7) with respiratory support	<100 (13.3) with respiratory support
Coagulation					
Platelets x10/mm^3	Normal	<150	<100	<50	<20
Liver					
Bilirubin, µmol/l (mg/dL)	Normal	20-32 (1.2-1.9)	33-101 (2.0-5.9)	102-204 (6.0-11.9)	<204 (12.0)
Cardiovascular					
Hypotension (mcg/kg/min)	Normal	MAP<70 mmHg	Any dose Dobutamine	Adrenaline <0.1 or Noradrenaline <0.1	Adrenaline >0.1 or Noradrenaline >0.1
Central Nervous System					
Glasgow Coma Score	Normal	13-14	10-12	6-9	<6
Renal					
Creatinine, µmol/l (mg/dL) or Urine output	Normal	110-170 (1.2-1.9)	171-299 (2.0-3.4)	300-440 (3.5-4.9) or <500 mL/day	>440 (5.0) or <200 mL/day

WESTERN CAPE CRITICAL CARE TRIAGE TOOL

Clinical Scores to be used for assessment

Clinical Frailty Scale

1 Very Fit – People who are robust, active, energetic and motivated. These people commonly exercise regularly. They are among the fittest for their age.

2 Well – People who have **no active disease symptoms** but are less fit than category 1. Often, they exercise or are very **active occasionally**, e.g. seasonally.

3 Managing Well – People whose **medical problems are well controlled,** but are **not regularly active** beyond routine walking.

4 Vulnerable – While **not dependent** on others for daily help, often **symptoms limit activities.** A common complaint is being "slowed up", and/or being tired during the day.

5 Mildly Frail – These people often have **more evident slowing,** and need help in **high order IADLs** (finances, transportation, heavy housework, medications). Typically, mild frailty progressively impairs shopping and walking outside alone, meal preparation and housework.

6 Moderately Frail – People need help with **all outside activities** and with **keeping house.** Inside, they often have problems with stairs and need **help with bathing** and might need minimal assistance (cuing, standby) with dressing.

7 Severely Frail – Completely dependent for personal care, from whatever cause (physical or cognitive). Even so, they seem stable and not at high risk of dying (within ~ 6 months).

8 Very Severely Frail – Completely dependent, approaching the end of life. Typically, they could not recover even from a minor illness.

9 Terminally Ill - Approaching the end of life. This category applies to people with **a life expectancy <6 months**, who are **not otherwise evidently frail.**

Scoring frailty in people with dementia

The degree of frailty corresponds to the degree of dementia. Common **symptoms in mild dementia** include forgetting the details of a recent event, though still remembering the event itself, repeating the same question/story and social withdrawal.

In **moderate dementia**, recent memory is very impaired, even though they seemingly can remember their past life events well. They can do personal care with prompting.

In **severe dementia**, they cannot do personal care without help.

mMRC Breathlessness Scale

This score should be used for patients diagnosed with COPD

Grade	Grade Description of Breathlessness
0	I only get breathless with strenuous exercise
1	I get short of breath when hurrying on level ground or walking up a slight hill
2	On level ground, I walk slower than people of the same age because of breathlessness, or have to stop for breath when walking at my own pace
3	I stop for breath after walking about 100 yards or after a few minutes on level ground
4	I am too breathless to leave the house or I am breathless when dressing

New York Heart Association (NYHA)

This score should be used for patients diagnosed with heart failure

Class	Patient symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath).
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath).
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases

ECOG Performance Status

This score should be used for patients diagnosed with a malignancy

Class	Patient symptoms
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair

Acknowledgements:

- Critical Care Society of South Africa: Allocation of Scarce Critical Care Resources During the COVID-19 Public Health Emergency in South Africa. - April 2020
- Ventilator allocation guidelines. Albany: New York State Task Force on Life and the Law, New York State Department of Health, November 2015
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- Canadian study on Health and Ageing, Revised 2008
- K. Rockwood et al. A Global clinical measure of fitness and frailty in elderly people. CMAJ 2005; 173:489-495
- Adapted from Vincent JL, de Mendonça A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on 'sepsis-related problems' of the European Society of Intensive Care Medicine. Crit Care Med. 1998;26(11):1793–1800
- Adapted from Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am. J. Clin. Oncol. 1982; 5 (6): 649–55
- Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnea. Chest. 1988;93(3):580-6

Supplementary methods: Telephonic Screening Instrument use

World Health Organization: Self-Reporting Questionnaire-20 (WHO SRQ-20)

SELF-REPORTING QUESTIONNAIRE (SRQ)
NTD TOOLKIT – Body functions and structures
Participant ID number: _____

The following questions are related to certain pains and problems, that may have bothered you in the last 30 days. If you think the question applies to you and you had to describe the problem in the last 30 days, answer YES. On the other hand, if the question does not apply to you and you did not have the problem in the last 30 days, answer NO.

1. Do you often have headaches?	Yes (1)	No (0)
2. Is your appetite poor?	Yes (1)	No (0)
3. Do you sleep badly?	Yes (1)	No (0)
4. Are you easily frightened?	Yes (1)	No (0)
5. Do your hands shake?	Yes (1)	No (0)
6. Do you feel nervous, tense or worried?	Yes (1)	No (0)
7. Is your digestion poor?	Yes (1)	No (0)
8. Do you have trouble thinking clearly?	Yes (1)	No (0)
9. Do you feel unhappy?	Yes (1)	No (0)
10. Do you cry more than usual?	Yes (1)	No (0)
11. Do you find it difficult to enjoy your daily activities?	Yes (1)	No (0)
12. Do you find it difficult to make decisions?	Yes (1)	No (0)
13. Is your daily work suffering?	Yes (1)	No (0)
14. Are you unable to play a useful part in life?	Yes (1)	No (0)
15. Have you lost interest in things?	Yes (1)	No (0)
16. Do you feel that you are a worthless person?	Yes (1)	No (0)
17. Has the thought of ending your life been on your mind?	Yes (1)	No (0)
18. Do you feel tired all the time?	Yes (1)	No (0)
19. Do you have uncomfortable feelings in your stomach?	Yes (1)	No (0)
20. Are you easily tired?	Yes (1)	No (0)

Duration of interview:_____ minutes

The WHO SRQ-20 measures non-specific psychological distress (60). Developed by the WHO, the test is in the Public Domain. The WHO-SRQ-20 has been validated for use in LMIC (60). Types of symptoms enquired about include anhedonia, worry, suicidality, neurovegetative and somatic symptoms (60). Answers are in a yes-no format, and various cut-offs had been recommended. We opted for the sensitive cut-off of 4 or more for males and 6 or more for females (60). Participants who answered "yes" to "has the thought of ending your life been on your mind?" were administered the Columbia Suicide Severity Rating Scale to detect imminent suicidality and to estimate suicide risk. These participants were then referred via the appropriate channels. The C-SSRS is in the Public Domain.

Columbia Suicide Severity Rating Scale (C-SSRS)

	Past 1 Month	
1) Have you wished you were dead or wished you could go to sleep and not wake up?		
2) Have you actually had any thoughts about killing yourself?		
If **YES** to 2, answer questions 3, 4, 5 and 6 If **NO** to 2, go directly to question 6		
3) Have you thought about how you might do this?		
4) Have you had any intention of acting on these thoughts of killing yourself, as opposed to you have the thoughts but you definitely would not act on them?	**High Risk**	
5) Have you started to work out or worked out the details of how to kill yourself? Did you intend to carry out this plan?	**High Risk**	
Always Ask Question 6	Life-time	Past 3 Months
6) Have you done anything, started to do anything, or prepared to do anything to end your life? *Examples*: Collected pills, obtained a gun, gave away valuables, wrote a will or suicide note, held a gun but changed your mind, cut yourself, tried to hang yourself, etc.		**High Risk**

NATIONAL SUICIDE PREVENTION LIFELINE
1-800-273-TALK (8255)
suicidepreventionlifeline.org

Any YES indicates that someone should seek behavioral healthcare.
However, if the answer to 4, 5 or 6 is YES, seek immediate help: go to the emergency room, call 1-800-273-8255, text 741741 or call 911. STAY WITH THEM until they can be evaluated.

Download Columbia Protocol app

Telephonic Montreal Cognitive Assessment (T-MoCA)

MONTREAL COGNITIVE ASSESSMENT / MoCA-BLIND
Version 7.1 Original Version

Name:
Education:
Sex:
Date of birth:
Date:

MEMORY		FACE	VELVET	CHURCH	DAISY	RED	POINTS
Read list of words, subject must repeat them. Do 2 trials even if 1st trial is successful. Do a recall after 5 minutes.	1st trial						No points
	2nd trial						

ATTENTION

Read list of digits (1 digit/sec.) Subject has to repeat them in the forward order [ ] 2 1 8 5 4
Subject has to repeat them in the backward order [ ] 7 4 2 ___/ 2

Read list of letters. The subject must tap with his hand at each letter A. No point if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B ___/ 1

Serial 7 subtraction starting at 100
[ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** ___/ 3

LANGUAGE

Repeat: I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ] ___/ 2

Fluency / Name maximum number of words in one minute that begin with the letter F.
[ ] _____ (N ≥ 11 words) ___/ 1

ABSTRACTION

Similarity between e.g. banana - orange = fruit
[ ] train - bicycle
[ ] watch - ruler ___/ 2

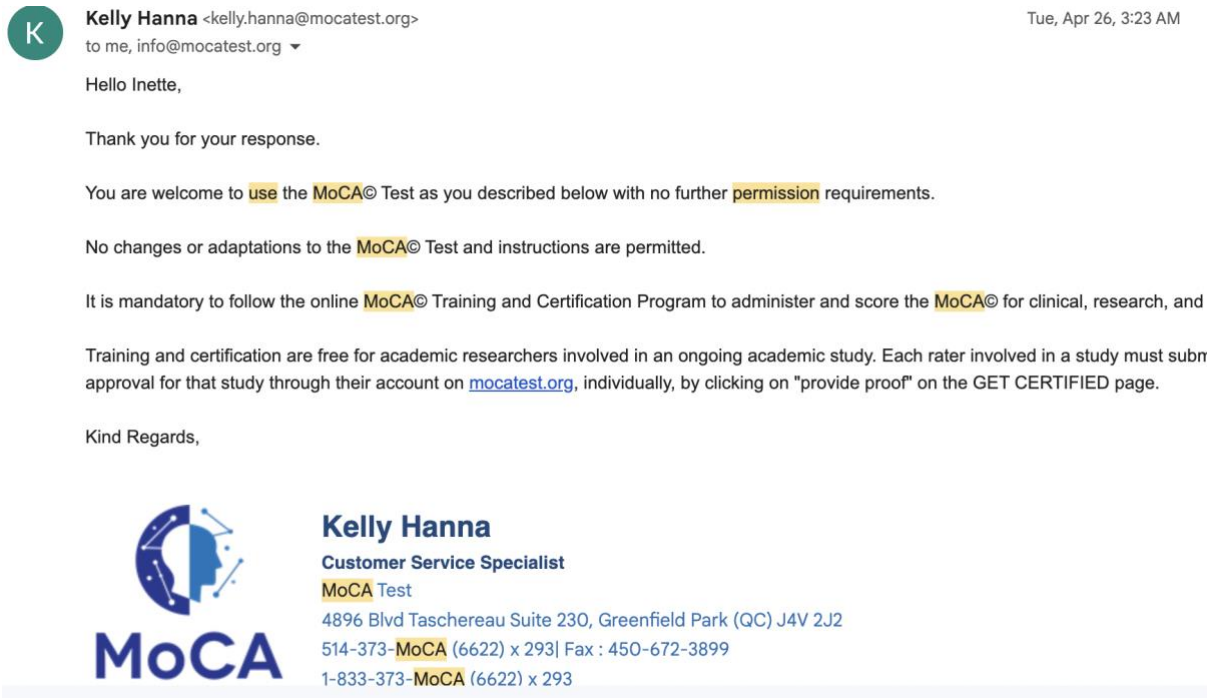
DELAYED RECALL	Has to recall words	FACE	VELVET	CHURCH	DAISY	RED	Points for UNCUED recall only	POINTS
	With no cue	[ ]	[ ]	[ ]	[ ]	[ ]		
Optional	Category cue							___/ 5
	Multiple choice cue							

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City ___/ 6

© Z. Nasreddine MD **www.mocatest.org** Normal ≥ 18 / 22 TOTAL ___/ 22
Add 1 point if ≤ 12 yr edu

Administered by:_____

Objective cognitive and memory results were obtained through administration of the T-MoCA. Permission for use was obtained from the developers of the MoCA test and the researchers administering the test both completed certification to administer and score this test.



Kelly Hanna <kelly.hanna@mocatest.org> Tue, Apr 26, 3:23 AM
to me, info@mocatest.org

Hello Inette,

Thank you for your response.

You are welcome to use the MoCA© Test as you described below with no further permission requirements.

No changes or adaptations to the MoCA© Test and instructions are permitted.

It is mandatory to follow the online MoCA© Training and Certification Program to administer and score the MoCA© for clinical, research, and

Training and certification are free for academic researchers involved in an ongoing academic study. Each rater involved in a study must subm approval for that study through their account on mocatest.org, individually, by clicking on "provide proof" on the GET CERTIFIED page.

Kind Regards,

MoCA

Kelly Hanna
Customer Service Specialist
MoCA Test
4896 Blvd Taschereau Suite 230, Greenfield Park (QC) J4V 2J2
514-373-MoCA (6622) x 293| Fax : 450-672-3899
1-833-373-MoCA (6622) x 293

The test is scored out of a total of 22, an extra mark is added for any participant with 12 or fewer years of schooling. 93 participants' test results are reported. Domains covered include learning and memory, complex attention, language, and some executive functioning. Phonemic fluency (Figure 3g), namely a participant's ability to generate words starting with a specific letter of the alphabet given 1 minute time, is one of the measures on the T-MoCA. Participants passed if they were able to generate 11 or more words in one minute. Specific rules apply regarding the type of words deserving a mark. Binary cut-offs were used, classifying 18 or more as normal, and less than 18 as impaired (61). Pendelbury et al report optimal cut-off on Tele-MoCA to be 18/19. Using 19 cut-off gave far more than half of participants affected (61). Concerns that a cut-off of 19/22 could possibly overestimate pathology and the fact that our population mostly utilised services at a Public Hospital and were from less fortunate socio-economic circumstances, the more conservative cut-off point 18/22 was selected. Two participants in the NIAID 2-3 category declined, and one in NIAID 4-6. One was due to language barrier affecting results. Permission for use was obtained from the developer of the MoCA.

Chalder Fatigue Scale-11 (CFS-11)

chalder fatigue scale

name: _____ date: _____

We would like to know more about any problems you have had with feeling tired, weak or lacking in energy in the last month. Please answer ALL the questions by ticking the answer which applies to you most closely. If you have been feeling tired for a long while, then compare yourself to how you felt when you were last well. Please tick only one box per line.

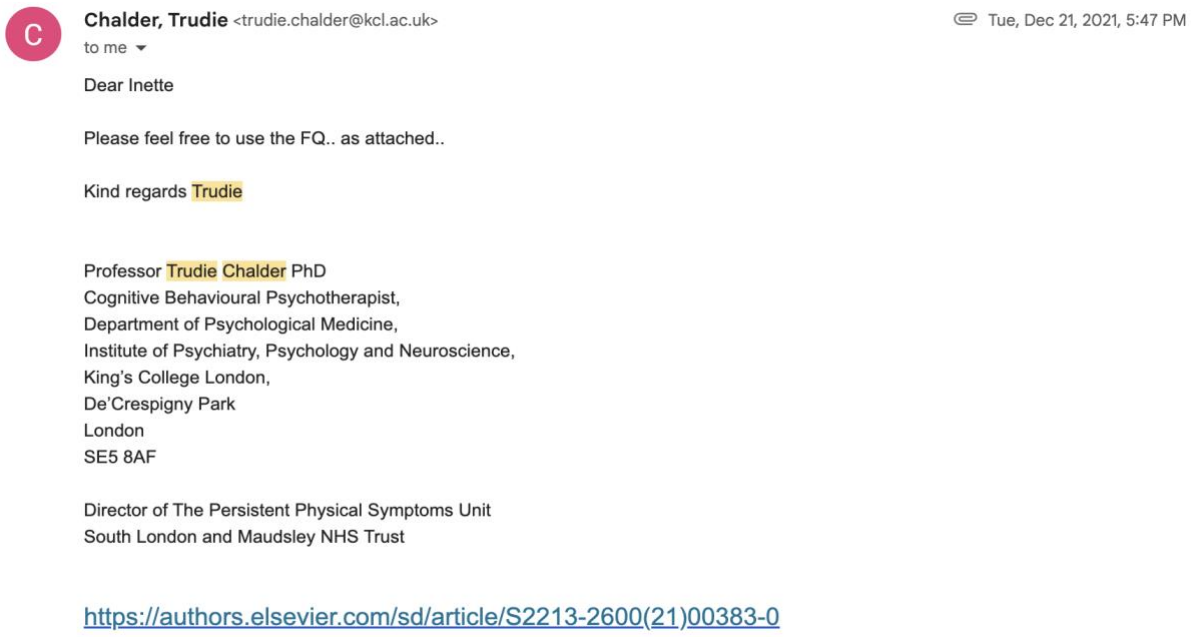
	less than usual	no more than usual	more than usual	much more than usual
do you have problems with tiredness?				
do you need to rest more?				
do you feel sleepy or drowsy?				
do you have problems starting things?				
do you lack energy?				
do you have less strength in your muscles?				
do you feel weak?				
do you have difficulties concentrating?				
do you make slips of the tongue when speaking?				
do you find it more difficult to find the right word?				
	better than usual	no worse than usual	worse than usual	much worse than usual
how is your memory?				

This scale can be scored "bimodally" with columns representing 0, 0, 1 & 1 and a range from 0 to 11 with a total of 4 or more qualifying for "caseness". Alternatively it can be scored in "Likert" style 0, 1, 2 & 3 with a range from 0 to 33. Mean "bimodal" score for CFS sufferers was 9.14 (SD 2.73) and for a community sample 3.27 (SD 3.21). Mean "Likert" score was 24.4 (SD 5.8) and 14.2 (SD 4.6).

total (0-33) =

Cella, M. and T. Chalder (2010). *"Measuring fatigue in clinical and community settings."* J Psychosom Res 69(1): 17-22. This study involved 361 CFS sufferers and 1615 individuals from the community. Average age was in the 30's. Fatigue levels were similar for males and females. A score of 29 discriminated between CFS sufferers and the community sample in 96% of cases and a score in the 30's discriminated in 100% of cases. The CFS sufferers also scored a mean of 26.99 on the Work & Social Adjustment Scale (W&SAS) with a SD of 8.6 (i.e. about 70% scoring between 18.4 and 35.6).

The Chalder Fatigue Scale (CFQ-11) has been used in multiple studies investigating for post-COVID symptoms (62-65). It contains 11 items and measures both physical as well as mental fatigue (62, 66). Permission for use was obtained from Prof Trudie Chalder.



Chalder, Trudie <trudie.chalder@kcl.ac.uk> Tue, Dec 21, 2021, 5:47 PM
to me

Dear Inette

Please feel free to use the FQ.. as attached..

Kind regards Trudie

Professor Trudie Chalder PhD
Cognitive Behavioural Psychotherapist,
Department of Psychological Medicine,
Institute of Psychiatry, Psychology and Neuroscience,
King's College London,
De'Crespigny Park
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Director of The Persistent Physical Symptoms Unit
South London and Maudsley NHS Trust

https://authors.elsevier.com/sd/article/S2213-2600(21)00383-0

The last three questions on the CFS-11 deal specifically with memory, word-finding and slips-of-tongue problems. These answers and data collected in the Case Report Forms were collated for subjective memory and cognitive findings, as opposed to objective findings obtained from the Tele-MoCA. The 22-point Tele-MoCA has been recommended for use in Covid-19 study populations (24). The Tele-MoCA has recently been validated in an ethnically and socio-economically diverse cohort in the United States (22). Adaptation to telephonic use have necessitated abbreviation of the executive function domain, and exclusion of visuospatial domain, and the recommended cut-off is 18 or 19 (61).

Generalized Anxiety Disorder-7 (GAD-7) Scale

GAD-7 Anxiety

Over the last two weeks, how often have you been bothered by the following problems?	Not at all	Several days	More than half the days	Nearly every day
1. Feeling nervous, anxious, or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Trouble relaxing	0	1	2	3
5. Being so restless that it is hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid, as if something awful might happen	0	1	2	3

Column totals _____ + _____ + _____ + _____ =

Total score _____

If you checked any problems, how difficult have they made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	Somewhat difficult	Very difficult	Extremely difficult
☐	☐	☐	☐

Source: Primary Care Evaluation of Mental Disorders Patient Health Questionnaire (PRIME-MD-PHQ). The PHQ was developed by Drs. Robert L. Spitzer, Janet B.W. Williams, Kurt Kroenke, and colleagues. For research information, contact Dr. Spitzer at ris8@columbia.edu. PRIME-MD® is a trademark of Pfizer Inc.

Scoring GAD-7 Anxiety Severity

This is calculated by assigning scores of 0, 1, 2, and 3 to the response categories, respectively, of "not at all," "several days," "more than half the days," and "nearly every day."
GAD-7 total score for the seven items ranges from 0 to 21.

0–4: minimal anxiety

5–9: mild anxiety

10–14: moderate anxiety

15–21: severe anxiety

The GAD-7 measures generalized anxiety symptoms and is in the public domain. Symptoms covered include restlessness, worry, irritability and feeling afraid. A score between 5-9 indicates mild anxiety, 10 or more is moderate anxiety. We used a cut-off as 8 or more for participants identified as having anxiety. It has been validated for use in LMIC's (60).

In addition to demographic and clinical data already available for each participant from the time of initial infection, an additional case report form was completed for each participant at the time of post-COVID telephonic interviewing (see below).

Case Report Form: Telephonic Screen

Case Report Form: Telephonic Screen
Covid19 Anxiety and Fatigue_Mental Health_CRF_V2_15/2/2022

Folder Number: ☐

Study Number: ☐ Telephonic Contact Start: ____ : ____ am / pm
End: ____ : ____ am / pm

Date (DD / MM / YY): ____ / ____ / ____

Demographics Contact No.: _____ Email: _____

Weight: _____ kg Height: _____ meters BMI Comment: _____

Has the participant's weight changed much since baseline? _____

Current smoker: ☐ No: Date stopped ____ / ____ / ____ ☐ Yes: Pack-years: _____

Indicate the wave(s) during which the participant became infected with Covid19?

☐ 1st *Mar 2020 -Jul 2020* ☐ 2nd *Dec 2020 - Feb 2021* ☐ 3rd *Jun 2021 - Aug 2021*

Have you been diagnosed with Covid19 more than once? ☐ No ☐ Yes

If yes, please specify: _____

Mental Health History

Have you ever struggled with mental health problems prior to being diagnosed with Covid19? ☐ No ☐ Yes

If yes, please specify: _____

Have you ever been diagnosed with a mental health problem or illness by any doctor? ☐ No ☐ Yes

If yes, please specify: _____

(if patient does not know their diagnosis, say "for example depression, bipolar disorder, anxiety, PTSD, OCD, psychosis, substance-induced psychosis, or Schizophrenia?")

Have you ever seen a Psychiatrist or Psychologist prior to being diagnosed with Covid19? ☐ No ☐ Yes

If yes, please specify: _____

Have you ever been prescribed medication for mental health reasons prior to your Covid19 diagnosis? For example, Prozac ☐ No ☐ Yes

If yes, please specify: _____

Before you had Covid19, have you ever had a period of at least 6 weeks when you were so tired that you could not do your usual duties? ☐ No ☐ Yes

If yes, please specify: _____

Did a doctor ever diagnose what the problem was?

_____

Post Covid19 Mental Health

Since your Covid19 infection, have you struggled with any mental health problems? ☐ No ☐ Yes

If yes, please specify: _____

Since your Covid19 infection, have you been diagnosed with a mental health problem or illness by any doctor? ☐ No ☐ Yes

If yes, please specify: _____

Since your Covid19 infection, have you seen a Psychiatrist or Psychologist? ☐ No ☐ Yes

If yes, please specify: _____

Since you had Covid19, were you prescribed any new psychiatric medication by a doctor? ☐ No ☐ Yes

If yes, please specify: _____

Are you currently using any psychiatric medication(s)? ☐ No ☐ Yes

If yes, which medication? _____

Start date: ____ / ____ / ____

_____

Start date: ____ / ____ / ____

P

Family History

Has anyone in your familiy been diagnosed with psychiatric problems such as anxiety; PTSD; depression; bipolar disorder or schizophrenia?

Relative 1: ☐ Problem: ☐

Relative 2: ☐ Problem: ☐

Relative 3: ☐ Problem: ☐

Relative 4: ☐ Problem: ☐

Covid19 Sequelae

Since you had Covid19, have you experienced any new symptoms, including physical or mental problems? ☐ No ☐ Yes

If yes, please specify: _____

_____

When did this start? _____

And are you still experiencing the problem? ☐ No ☐ Yes

_____

Have you ever lost your sense of taste or smell? ☐ No ☐ Yes

If yes, when: _____

Has it recovered since? ☐ No ☐ Yes

Have you had headaches during or after your COVID-19 infection? ☐ No ☐ Yes

If yes, when did it start: _____

Did you suffer from frequent headaches before you had Covid19? ☐ No ☐ Yes

Do you still suffer from frequent headaches? ☐ No ☐ Yes

Did you have your Covid19 vaccination? ☐ No ☐ Yes

Which? ☐ Pfizer ☐ AstraZeneca ☐ Johnson & Johnson ☐ Other

When?: _____

Did you have any lingering symptoms after your vaccination? ☐ No ☐ Yes

If yes, please specify: _____

Additional Questions to Cover Missing Information

_____

_____

_____

_____

_____

_____

Table S2: Included analytes from the O-link inflammatory and cardiovascular panels

4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1
ADA	Adenosine Deaminase
AXIN1	AXIN1
CASP8	Caspase-8
CCL11	Eotaxin
CCL19	C-C motif chemokine 19
CCL20	C-C motif chemokine 20
CCL23	C-C motif chemokine 23
CCL25	C-C motif chemokine 25
CCL28	C-C motif chemokine 28
CCL3	C-C motif chemokine 3
CCL4	C-C motif chemokine 4
CD244	Natural killer cell receptor 2B4
CD40	Cluster of differentiation 40
CD5	T-cell surface glycoprotein CD5
CD6	T cell surface glycoprotein CD6 isoform
CD8A	T-cell surface glycoprotein CD8 alpha chain
CDCP1	CUB domain-containing protein 1
CSF-1	Macrophage colony-stimulating factor 1
CST5	Cystatin D
CX3CL1	Fractalkine
CXCL1	C-X-C motif chemokine 1
CXCL10	C-X-C motif chemokine 10
CXCL11	C-X-C motif chemokine 11
CXCL5	C-X-C motif chemokine 5
CXCL6	C-X-C motif chemokine 6
CXCL9	C-X-C motif chemokine 9
DNER	Delta and Notch-like epidermal growth factor-related receptor
EN-RAGE	Protein S100-A12
FGF-19	Fibroblast growth factor 19
FGF-21	Fibroblast growth factor 21
FGF-5	Fibroblast growth factor 5
Flt3L	Fms-related tyrosine kinase 3 ligand
GDNF	Glial cell-line-derived neurotrophic factor
HGF	Hepatocyte growth factor
IFN-gamma	Interferon gamma
IL-10RA	Interleukin-10 receptor subunit alpha
IL-10RB	Interleukin-10 receptor subunit beta
IL-12B	Interleukin-12 subunit beta
IL-17A	Interleukin 17A
IL-17C	Interleukin-17C
IL-18R1	Interleukin 18 receptor 1
IL10	Interleukin 10
IL18	Interleukin 18
IL6	Interleukin 6
IL7	Interleukin 7

IL8	Interleukin 8
LAP TGF-beta-1	Latency-associated peptide transforming growth factor beta-1
LIF-R	Leukaemia inhibitory factor receptor
MCP-1	Monocyte chemotactic protein 1
MCP-2	Monocyte chemotactic protein 2
MCP-3	Monocyte chemotactic protein 3
MCP-4	Monocyte chemotactic protein 4
MMP-1	Matrix metalloproteinase-1
MMP-10	Matrix metalloproteinase-10
NT-3	Neurotrophin-3
OPG	Osteoprotegerin
OSM	Oncostatin-M
PD-L1	Programmed cell death 1 ligand 1
SCF	Stem cell factor
SIRT2	SIR2-like protein 2
SLAMF1	Signalling lymphocytic activation molecule
ST1A1	Sulfotransferase 1A1
STAMBP	STAM-binding protein
TGF-alpha	Transforming growth factor alpha
TNF	Tumour necrosis factor
TNFB	Tumour necrosis factor-beta
TNFRSF9	Tumour necrosis factor receptor superfamily member 9
TNFSF14	Tumour necrosis factor ligand superfamily member 14
TRAIL	TNF-related apoptosis-induced ligand
TRANCE	TNF-related activation-induced cytokine
TSLP	Thymic stromal lymphopoietin
TWEAK	Tumour necrosis factor (Ligand) superfamily, member 12
VEGF-A	Vascular endothelial growth factor A
uPA	Urokinase-type plasminogen activator
ANG-1	Angiopoietin-1
ADM	Adrenomedullin
SRC	Proto-oncogene tyrosine-protein kinase Src
IL-1ra	Interleukin-1 receptor antagonist protein
PRSS27	Serine protease 27
PDGF subunit B	Platelet-derived growth factor B
Gal-9	Galectin-9
RAGE	Receptor for advanced glycosylation end products
SOD2	Superoxide dismutase [Mn], mitochondrial
CTRC	Chymotrypsin C
SPON2	Spondin-2
GH	Growth hormone
FS	Follistatin
SERPINA12	Serpin A12
SORT1	Sortilin
CCL17	C-C motif chemokine 17
MMP7	Matrix metalloproteinase-7
Dkk-1	Dickkopf-related protein 1

PRSS8	Prostasin
CTSL1	Cathepsin L1
HSP27	Heat shock 27 kDa protein

Supplementary methods: Laboratory methods

Blood sampling

Once recruited under the UCT FHS COVID-19 Biorepository, blood sampling of participants occurred at every study visit timepoint: one 10ml SST tube (relevant to this study), centrifuged at 1300G for 15 minutes at room temperature. Samples, once aliquoted, were stored long-term at -80^{o}C.

Laboratory methods

Biosafety

All work with samples from COVID-19 patients, prior to viral inactivation, was conducted in a BSL-2 biosafety cabinet in an approved facility at the University of Cape Town (UCT) Faculty of Health Sciences (FHS). Viral inactivation of samples was performed by addition of 1% Triton X-100 to the sample buffer of assays conducted at UCT. Prior to commencement of work, both faculty and institutional level biosafety approval was obtained.

ACE1 activity measurement

The ACE1 activity assay was performed as previously described (56), with modifications for work in serum in a 96-well black microplate (Eppendorf: 0030601700). The 5x assay buffer consisted of 0.5M potassium phosphate, 1.5M NaCl and 10μM ZnSO4 (VWR MW:287.56 g/mol). The substrate used was Z-Phe-His-Leu (Bachem: 4000599) in 0.028 NaOH. Serum was diluted 1:5 in PBS, and assayed in triplicate. The fluorescence of the plate was measured at 360nm excitation and 485nm emission (Perkin Elmer EnSpire Multimode Plate Reader). From the results, a standard curve was generated in RStudio using the *drc* package, and ACE1 activity (nmol/min/mL) was then calculated by dividing the amount of HL produced by incubation time and correcting for serum volume and serum dilution.

The overall intra-assay CV was 4.1% (standard deviation 1.32%), and the inter-assay CV was 3.45%.

ACE2 activity measurement

The serum ACE2 activity assay as published by Fagyas, et al (67) was modified as follows: The assay buffer was MES-based (125mM 2-(N-Morpholino)ethanesulfonic acid (MES), 4μM $ZnCl_2$, pH 6.5), and 20μM MLN-4760 (MedChemExpress: HY-19414) was used as a specific ACE2 inhibitor, and the substrate-salt solution consisted of 20μM Mca-APK(Dnp)-OH TFA

(Sigma-Aldrich: SML2948) with 600mM NaCl. The fluorescence of the microplate was read continuously for 1 hour at 37°C, 500rpm shaking, 320nm excitation and 405nm emission (Perkin Elmer EnSpire Multimode Plate Reader). ACE2 activity (pmol/min/mL) was calculated in RStudio by dividing the median slope (with a rolling mean applied) of each sample by the slope of the standard curve (r^2 >0.99 in all cases), corrected for serum volume. The overall intra-assay CV was 5.25% (standard deviation 1.82%), and the inter-assay CV was 5.04%

RAS Fingerprinting®

The concentrations of the RAS peptides angiotensin (Ang) I, Ang II, Ang 1-7, and Ang 1-5 were measured at an ex vivo equilibrium state in serum. This was done using RAS Fingerprinting® (Attoquant Diagnostics) which employs liquid chromatography-tandem mass spectrometry (LC-MS/MS), as previously described (68), with the following modifications:
The lower limit of detection for Ang I, Ang II, Ang 1-7, Ang 1-5, in serum at equilibrium was 3 pmol/L, 2 pmol/L, 3 pmol/L, and 2 pmol/L, respectively. The surrogate markers for enzyme activity used in this study were i) for ACE1 function (ACE-S = Ang II/Ang I), and ii) for ACE2 function (Ang 1-5/Ang II).

SARS-CoV-2 antibody measurements

SARS-CoV-2 anti-spike and anti-nucleocapsid protein antibody titres in serum were determined using an ELISA method (National Institute for Communicable Diseases (NICD)), while neutralising antibody titres were measured using a SARS-CoV-2 pseudovirus-based neutralisation assay (NICD), both as previously published (69). For anti-spike antibody and neutralising antibody titres, the spike protein variant used depended on the predominant circulating variant in South Africa at the time of sampling, or in the case of the neutralising assay, the confirmed SARS-CoV-2 variant of infection where sequenced.

Table S3A: Baseline characteristics of interviewed patients stratified by neuropsychiatric outcomes with P-values included

Characteristics	Complete Cohort, n=97	MOCA*			Subj**			GAD***			Fatigue****		
		Unimpaired, n=52	Impaired, n= 41	P-value	Unreported, n=46	Reported, n=51	P-value	Negative, n= 73	Positive, n=24	P-value	Negative, n=43	Positive, n=54	P-value
Age, mean(sd)	47.56 (11.26)	46.75 (12.40)	49.61 (9.53)	0.229	46.46 (11.51)	48.55 (11.06)	0.3636	48.37 (11.88)	45.08 (8.88)	0.2167	46.60 (12.00)	48.31 (10.72)	0.4604
Female, x(%)	52 (53.61)	25 (48.08)	25 (60.98)	0.215	24 (52.17)	28 (54.90)	0.788	38 (52.05)	14 (58.33)	0.593	24 (55.81)	28 (51.85)	0.697
COVID related													
Duration of symptoms prior to baseline sampling, days med(IQR)	7 (5 – 11)	7 (5 – 11)	8 (5 – 11)	0.3484	8 (5 – 12)	7 (5 – 10)	0.5586	7 (5-11)	7.5 (5 – 11)	0.7344	7.5 (5 – 11)	7 (5 – 11)	0.7675
Symptoms, n(%)													
- Cough	45 (46.39)	25 (48.08)	18 (43.90)	0.689	23 (50.00)	22 (43.14)	0.499	32 (43.84)	13 (54.17)	0.379	17 (39.53)	28 (51.85)	0.227
- Fever	16 (16.49)	10 (19.23)	6 (14.63)	0.560	8 (17.39)	8 (15.69)	0.821	12 (16.44)	4 (16.67)	0.979	8 (18.60)	8 (14.81)	0.617
- Myalgia	6 (6.19)	5 (9.62)	1 (2.44)	0.162	1 (2.17)	5 (9.80)	0.119	4 (5.48)	2 (8.33)	0.615	2 (4.65)	4 (7.41)	0.576
- Headache	6 (6.19)	4 (7.69)	2 (4.88)	0.583	3 (6.52)	3 (5.88)	0.896	2 (2.74)	4 (16.67)	0.014	2 (4.65)	4 (7.41)	0.576
- GIT symptoms	6 (6.19)	3 (5.77)	3 (7.32)	0.763	4 (8.70)	2 (3.92)	0.330	5 (6.85)	1 (4.17)	0.636	4 (9.30)	2 (3.70)	0.255

Pf ratio, med(IQR)	157 (74 – 270)	157 (74 – 252)	201.5 (74.5 – 276.5)	0.4117	75 (56 – 269)	180.5 (83 – 270)	0.3589	141.5 (70.5 – 246)	211 (77 - 310)	0.1232	174.5 (56 – 277)	150 (77 – 252)	0.8618
High flow nasal oxygen, x(%)	4 (4.65)	2 (4.17)	2 (5.88)	0.268	2 (5.13)	2 (4.26)	0.533	1 (1.54)	3 (14.29)	0.169	2 (5.41)	2 (4.08)	0.854
Duration of admission, med(IQR)	3 (2 – 5)	3 (2 - 5)	2 (2 - 4)	0.2541	3 (2 – 5)	2 (2 – 4)	0.1746	3 (2 – 5)	2.5 (1 – 5)	0.6639	2 (2 – 5)	3 (2 – 5)	0.8703
Medical co-morbidities													
Hypertension, x(%)	38 (39.18)	19 (36.54)	19 (46.34)	0.340	15 (32.61)	23 (45.10)	0.208	31 (42.47)	7 (29.17)	0.247	15 (34.88)	23 (42.59)	0.440
Diabetes Mellitus type 2, x(%)	27 (27.84)	13 (25.00)	13 (31.71)	0.474	11 (23.91)	16 (31.37)	0.413	21 (28.77)	6 (25.00)	0.721	12 (27.91)	15 (27.78)	0.989
HIV, x(%)	4 (4.12)	2 (3.85)	2 (4.88)	0.808	1 (2.17)	3 (5.88)	0.359	3 (4.11)	1 (4.17)	0.990	0 (0.00)	4 (7.41)	0.068
Admission Laboratory results[3]													
White cell count, (10^9/L), med(IQR)	8.68 (6.68 – 10.55)	8.96 (6.19 – 12.81)	8.55 (6.64 – 10.31)	0.5436	9.89 (6.64 – 11.91)	8.68 (6.68 – 10.55)	0.6967	8.84 (6.72 – 12.81)	8.60 (6.39 – 9.89)	0.3951	8.87 (6.64 – 10.55)	8.55 (6.68 – 10.86)	0.5046
Lymphocyte count, (10^9/L), med(IQR)	1.17 (0.72 – 1.54)	1.33 (0.77 – 1.6)	1.03 (0.91 – 1.5)	0.4989	1.19 (0.68 – 1.38)	1.1 (0.80 – 1.83)	0.3369	1.19 (0.79 – 1.5)	0.87 (0.61 – 1.73)	0.8441	1.24 (0.72 – 1.49)	0.97 (0.77 – 1.54)	0.7699
Haemoglobin, (g/dL), med(IQR)	13.5 (12.7 – 14.4)	13.85 (13 – 14.4)	13 (12.6 – 14.7)	0.3474	13.3 (12.4 – 13.9)	13.5 (12.8 – 14.6)	0.8881	13.3 (12.6 – 14.4)	13.5 (12.8 – 14.3)	0.3296	13.25 (12.4 – 14.2)	13.5 (12.8 – 14.4)	0.8682
D-dimer, (mg/L), med(IQR)	0.4 (0.27 – 0.67)	0.41 (0.28 – 0.86)	0.39 (0.21 – 0.67)	0.8901	0.39 (0.28 – 0.65)	0.41 (0.26 – 0.735)	0.2198	0.42 (0.38 – 0.8)	0.27 (0.16 – 0.42)	0.1334	0.41 (0.36 – 0.8)	0.39 (0.25 – 0.67)	0.9368
C reactive protein, (mg/L), med(IQR)	102 (61.5 - 150)	77 (45 – 131)	106.5 (70 – 152.5)	0.4776	68.5 (38 – 93)	122 (77 – 169)	0.032	102 (62 – 169)	102 (53 – 127)	0.5713	70 (45 – 104)	115.5 (74 – 169)	0.1075

Creatinine, (mmol/L), med(IQR)	73.5 (66 – 89)	78.5 (69 – 91)	71.5 (66 – 88.5)	0.5641	77 (66 – 93)	73 (66 – 89)	0.6944	77 (69 – 94)	68.5 (66 – 82)	0.1467	77 (67 – 92)	71 (66 – 88.5)	0.6503
Urea, (umol/L), med(IQR)	5.3 (4.1 – 7.1)	5.05 (4.1 – 7.1)	5.6 (4.05 -7)	0.5920	5.4 (4.1 – 7.3)	5.2 (3.8 – 6.7)	0.9987	5.3 (4.15 – 7.25)	5.35 (3.6 – 6.7)	0.4677	5.5 (4.1 – 6.6)	5.05 (3.85 – 7.1)	0.3807

ACE-I: Angiotensin converting enzyme inhibitor, ARB: angiotensin receptor antagonist, IQR: interquartile range

1. Description of the other co-morbidities
2. Description of chronic lung disease (eg asthma, COPD)
3. Dependent on clinical practice

*T-MoCA cut-off <18
** Subjective memory or cognitive problems reported on Case Report Form and/or last 3 questions of CFS-11
*** GAD-7 cut-off 8 or more
**** CFS cut-off 4 or more

Table S3B: Baseline characteristics of interviewed patients stratified by Alpha, Beta and Delta COVID waves

Characteristic	**Covid19 wave**			
	Alpha (1st), n= 31	**Beta (2nd), n= 34**	**Delta (3rd), n= 32**	**P-value**
Age, mean(sd)	47.12 (9.43)	48.74 (12.40)	46.72 (11.87)	0.7469
Female, x(%)	19 (61.29)	22 (64.71)	11 (34.38)	0.028
Covid19 Related				
Duration of symptoms prior to baseline sampling, days med(IQR)	7 (5 – 8)	9.5 (7 -13)	6.5 (5 – 11)	0.0392
Symptoms, n(%)				
- Cough	13 (41.94)	8 (23.53)	24 (75.00)	<0.001
- Fever	7 (22.58)	2 (5.88)	7 (21.88)	0.117
- Myalgia	2 (6.45)	0 (0.00)	4 (12.50)	0.108
- Headache	0 (0.00)	0 (0.00)	6 (18.75)	0.002
- GIT symptoms	0 (0.00)	3 (8.82)	3 (9.38)	0.222
Pf ratio, med(IQR)	226 (150 – 288)	92 (56 – 270)	111 (63 – 212.5)	0.0723
High flow nasal oxygen, x(%)	0 (0.00)	1 (3.70)	3 (10.00)	0.003
Duration of admission, med(IQR)	2 (2 -3)	3 (2 – 6)	4 (2 -5)	0.0098
Medical co-morbidities				
Hypertension, x(%)	12 (38.71)	13 (38.24)	13 (40.63)	0.978
Diabetes Mellitus type 2, x(%)	11 (35.48)	8 (23.53)	8 (25.00)	0.511
HIV, x(%)	1 (3.23)	2 (5.88)	1 (3.13)	0.815
Admission Laboratory results[3]				
White cell count, (10^9/L), med(IQR)	8.56 (5.89 – 10.01)	7.90 (6.66 – 10.28)	9.89 (7.23 – 12.81)	0.2869
Lymphocyte count, (10^9/L),, med(IQR)	1.39 (0.91 – 1.68)	1.19 (0.8 – 1.49)	0.68 (0.44 – 1.03)	0.0517
Haemoglobin, (g/dL), med(IQR)	13 (12.35 – 14.45)	13.3 (12.65 – 14.15)	13.9 (13.2 – 14.4)	0.1811
D-dimer, (mg/L), med(IQR)	0.33 (0.1 – 0.45)	0.41 (0.23 – 0.86)	0.41 (0.33 – 0.63)	0.5374
C reactive protein, (mg/L), med(IQR)	82.5 (31 – 169)	102 (63 – 127)	114.5 (77 – 168.5)	0.7930
Creatinine, (mmol/L), med(IQR)	71.5 (65.5 – 78.5)	81.5 (71.5 - 95)	66 (64 – 89)	0.1964
Urea, (umol/L), med(IQR)	4.4 (3.45 – 5.5)	4.9 (4.05 – 7.15)	6.35 (5.4 – 7.7)	0.3060

Table S4: Neuropsychiatric outcomes - differences between waves

Characteristic	1, N = 31[1]	2, N = 34[1]	3, N = 32[1]	p-value[2]
gad7_cutoff_pos_neg				0.7
No anxiety	25 (81%)	25 (74%)	23 (72%)	
Has anxiety	6 (19%)	9 (26%)	9 (28%)	
cfs_cutoff_pos_neg				0.8
No fatigue	15 (48%)	15 (44%)	13 (41%)	
Has fatigue	16 (52%)	19 (56%)	19 (59%)	
moca_final_binary				0.2
Impaired	17 (55%)	14 (44%)	10 (33%)	
Unimpaired	14 (45%)	18 (56%)	20 (67%)	
Unknown	0	2	2	
subj_poor_mem_cog_since_cov19				0.5
No impairment	12 (39%)	18 (53%)	16 (50%)	
Memory/Cognitive	19 (61%)	16 (47%)	16 (50%)	

[1]n (%)

[2]Pearson's Chi-squared test

Sensitivity analysis

Table S5A: Complement of total cohort compared to the post-COVID cohort

Characteristics	Total cohort (without post-COVID cohort), n= 125	Post-COVID cohort, n= 99	P-value
Age in years, mean(sd)	48.73 (14.58)	47.64 (11.19)	0.5268
Female, x(%)	69 (55.20)	54 (54.55)	1.0000
BMI, median (IQR) (51/125; 11/99 missing)	27.51 (25.12 – 34.59)	31.39 (26.88 – 37.56)	0.0532
Smoking status (13/125; 1/99 were missing)			
- Current smoker, n(%)	14 (12.50)	13 (13.27)	1.0000
- Previous smoker, n(%)	18 (16.07)	18 (18.37)	0.5812
COVID-19 Related			
Dominant variant, n(%)	(34/125 missing)	(21/99 missing)	
- Ancestral	24 (26.37)	27 (34.62)	0.2655
- Beta	40 (43.96)	27 (34.62)	0.2272
- Delta	27 (29.67)	24 (30.77)	0.9877
COVID-19 Severity, n(%)	(2/125 missing)	(1/99 missing)	
- Asymptomatic	24 (19.51)	15 (15.31)	0.5507
- Mild/Moderate Home	21 (17.07)	28 (28.57)	0.0769
- Moderate-Severe Hospitalized	34 (27.64)	33 (33.67)	0.4404
- Critical Hospitalized	44 (35.77)	22 (22.45)	0.0551
Medical co-morbidities			
Hypertension, x(%)	52 (41.60)	39 (39.39)	0.8616
Diabetes Mellitus type 2, x(%)	36 (28.80)	27 (27.27)	0.9335
HIV, x(%) (1/125 missing)	12 (9.68)	4 (4.08)	0.1988
Other, x(%) (1/125 missing)	51 (41.13)	37 (37.37)	0.6894

Table S5B: Complement of total cohort without deceased cohort, compared to the post-COVID cohort

Characteristics	Total cohort (without post-COVID or deceased cohort), n= 102	Post-COVID cohort, n= 99	P-value
Age in years, mean(sd)	47.43 (14.97)	47.64 (11.19)	0.9124
Female, x(%)	58 (56.86)	54 (54.55)	0.8521
BMI, median (IQR) (36/102; 11/99 missing)	28.38 (25.02 – 34.06)	31.39 (26.88 – 37.56)	0.0233
Smoking status (11/102; 1/99 were missing)			
- Current smoker, n(%)	13 (14.29)	13 (13.27)	0.9967
- Previous smoker, n(%)	13 (14.29)	18 (18.37)	0.5557
COVID-19 Related			
Dominant variant, n(%)	(26/102 missing)	(21/99 missing)	
- Ancestral	23 (30.26)	27 (34.62)	0.6118
- Beta	28 (36.84)	27 (34.62)	0.8571
- Delta	25 (32.89)	24 (30.77)	0.8650
COVID-19 Severity, n(%)	(2/102 missing)	(1/99 missing)	
- Asymptomatic	24 (24.00)	15 (15.31)	0.1712
- Mild/Moderate Home	21 (21.00)	28 (28.57)	0.2819
- Moderate-Severe Hospitalized	30 (30.00)	33 (33.67)	0.6853
- Critical Hospitalized	25 (25.00)	22 (22.45)	0.7967
Medical co-morbidities			
Hypertension, x(%)	40 (39.22)	39 (39.39)	1.0000
Diabetes Mellitus type 2, x(%)	28 (27.45)	27 (27.27)	1.0000
HIV, x(%) (1/102 missing)	11 (10.89)	4 (4.08)	0.1185
Other, x(%) (1/102 missing)	40 (39.60)	37 (37.37)	0.8581

Individual accounts of Subjective Memory Difficulties

Anomic aphasia, colloquially referred to as “word-finding difficulties”, was particularly well described. Some participants reported having been alerted by family members or employers to perceived changes in their cognitive performance. One reported that his 12- and 14-year-old children told him “Daddy you don't speak properly"; another participant reports noticing how he/she frequently started saying "that thingy" when unable to recall the correct word; further complaints included struggling to recall the names of objects or furniture; problems with interchanging words and calling objects by the wrong name; needing reminders of familiar people’s names; and calling her own child by the wrong name.

Further subjective descriptions of memory impairment include a former mathematician now “not able to hold two numbers” in her head; forgetting the content of conversations; forgetting where he put things and forgetting assignments; if interrupted while doing something cannot remember what was busy with; spending money and being unable to remember what it was spent on; forgetting appointments; forgetting well-known recipes; losing keys; forgetting glasses while wearing them; putting on a second mask over original mask; needing electronic documentation; needing to write everything down; and feeling “less sharp” after Covid.

Table S6: O-link sampling stratified by neuropsychiatric outcomes and disease severity groups

	Generalized Anxiety disorder 7	
Peak	No anxiety	Has anxiety
Asymptomatic	4	2
Mild-Moderate Home	21	2
Moderate-Severe Hospital	20	9
Critical	12	5
Recovery	55	18

	Chalder Fatigue	
Peak	No fatigue	Has fatigue
Asymptomatic	4	2
Mild-Moderate Home	11	12
Moderate-Severe Hospital	12	17
Critical	6	11
Recovery	33	40

	Montreal Cognitive Assessment	
Peak	Unimpaired	Impaired
Asymptomatic	3	3
Mild-Moderate Home	18	5
Moderate-Severe Hospital	13	15
Critical	8	7
Recovery	42	28

	Subjective cognitive assessment	
Peak	No impairment	Subjective cognitive impairment
Asymptomatic	5	1
Mild-Moderate Home	12	11
Moderate-Severe Hospital	10	19
Critical	10	7
Recovery	37	36

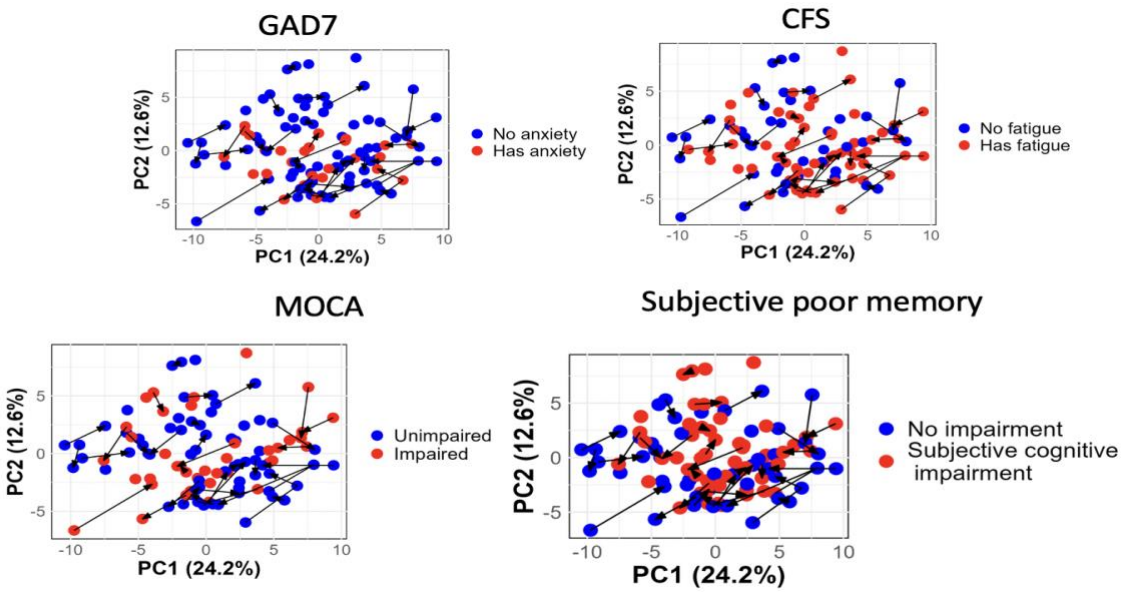

Figure S6A: PCA plot at Peak illness severity combining O-link inflammation, RAS and Antibody expression

a) PCA plot at peak illness severity combining O-link inflammatory, RAS, and Antibody panel results for participants with (red) or without (blue) anxiety post-COVID infection

b) PCA plot at peak illness severity combining O-link inflammatory, RAS, and Antibody panel results for participants with (red) or without (blue) fatigue post-COVID infection

c) PCA plot at peak illness severity combining O-link inflammatory, RAS, and Antibody panel results for participants with or without impaired total Tele-MoCA scores post-COVID infection

d) PCA plot at peak illness severity combining O-link inflammatory, RAS, and Antibody panel results for participants with or without subjectively impaired memory post-COVID infection

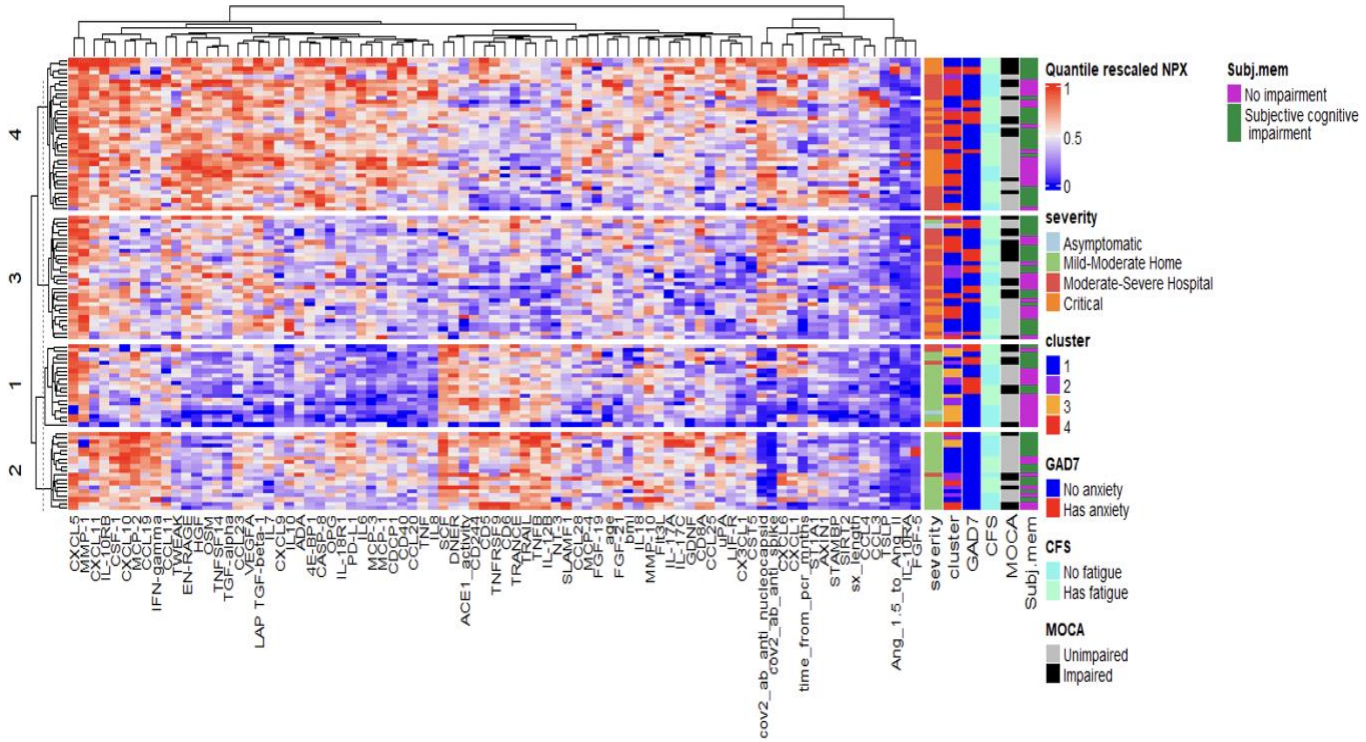

Figure S6B: Heatmap at Peak combining O-link inflammation, RAS and Antibody expression

* Rows represent study participants, showing expression of different proteins in each participant

** Each protein and analyte are represented in different columns

*** On the far right, columns represent severity groups

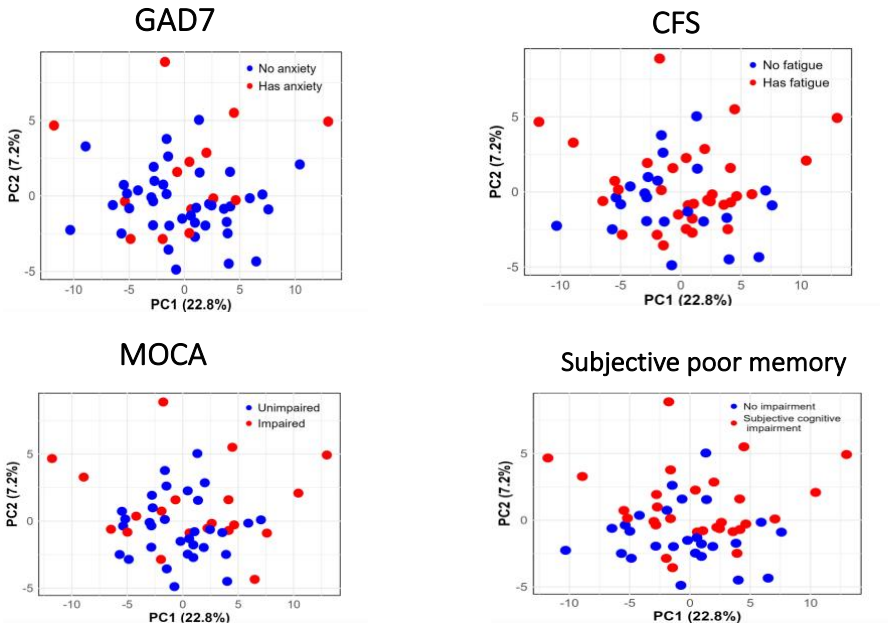

Figure S7A: PCA plot of recovery samples

a) PCA plot depicting O-link inflammatory panel results at recovery after acute infection for participants with (red) or without (blue) anxiety post-COVID infection

b) PCA plot depicting O-link inflammatory panel results at recovery after acute infection for participants with (red) or without (blue) fatigue post-COVID infection

c) PCA plot depicting O-link inflammatory panel results at recovery after acute infection for participants with or without impaired total Tele-MoCA scores post-COVID infection

d) PCA plot depicting O-link inflammatory panel results at recovery after acute infection for participants with or without subjectively impaired memory post-COVID infection

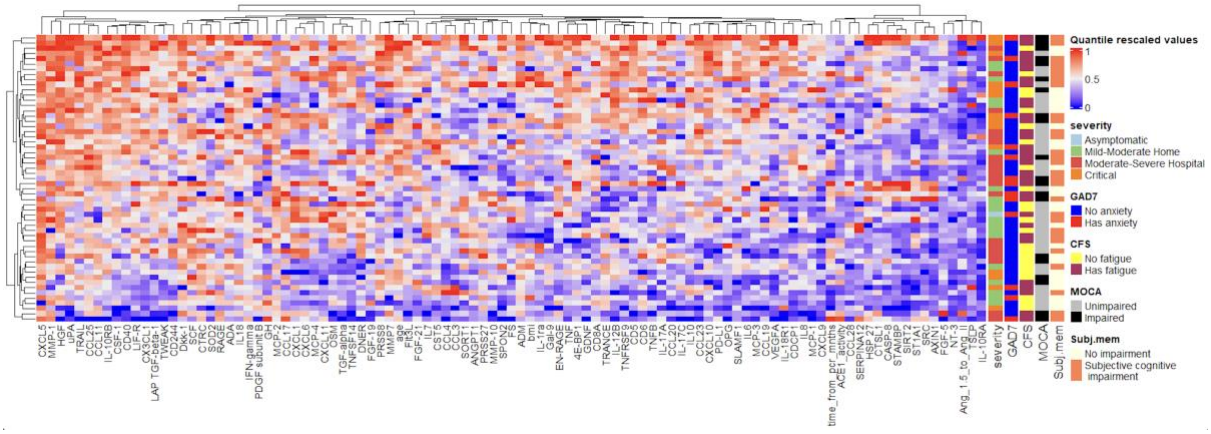

Figure S7B: Heatmap of Recovery samples

* Rows represent study participants, showing expression of different proteins in each participant

** Each protein and analyte are represented in different columns

*** On the far right, columns represent severity groups

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Additional MMed appendices

Appendix A: Informed Consent

Consent form for telephonic interview
Post Covid19 anxiety and fatigue in a cohort of South African patients
Principle Investigator: A/Prof J Peter

Introduction

You are being invited to take part in a research project. You are contacted because you enrolled in the UCT FHS COVID19 biorepository (PI: Prof Ntobeko Ntusi; HREC R021/2020) at Groote Schuur Hospital. I will explain the purpose of this study to you and give you an opportunity to ask questions before you make any decision. Your participation is entirely voluntary and will not affect your participation in the original study or any ongoing care you are receiving.

This study has been submitted for approval to the **Human Research Ethics Committee** of the Faculty of Health Sciences of the University of Cape Town and will be conducted according to the ethical guidelines and principles of the International Declaration of Helsinki and the South African Guidelines for Good Clinical Practice.

I am _____(full name of delegated study staff member) from the University of Cape Town. This study is being conducted jointly by the Divisions of Psychiatry and Allergology and Clinical Immunology at UCT, the supervisors are Prof Dan Stein, Head of the Department of Psychiatry at UCT, and A/Prof Jonathan Peter, Unit Head of the Lung Institute and Head of the Division of Allergology and Clinical Immunology at UCT.

What is this research about?

Through observing patients' experiences after they recover from Covid19, it became evident that many people recover fully without experiencing any ongoing symptoms, while some people report noticing ongoing problems with their health, such as severe fatigue. We are running a study linked to the study you agreed to participate in, and we are now looking at whether patients admitting to hospital with COVID19 are still experiencing symptoms of ongoing fatigue or anxiety persisting for 3 months or longer after their hospitalization with COVID19.

We are interested in your unique experience, and we may want to look at samples taken when and after you were ill. Through matching your experience and your biological samples we could potentially learn new things about the virus and how it affects the human body and brain.

What will your responsibilities be?

We would like to ask you brief simple screening questions over the phone. This should not take more than 30 minutes of your time. Questions are about your energy levels, sleep, appetite, your mood, feelings of stress, and physical problems. Thereafter a short tele-MoCA (Montreal Cognitive screen) will follow.

Are there risks involved in your taking part in this study?

Talking about a person's health and symptoms can make a person feel upset or uncomfortable. If you need treatment, we will refer you for specialized medical help. If you are experiencing thoughts about suicide, you will also immediately be referred for professional help.

Will you benefit from taking part in this research?

There are no direct personal benefits associated with your participation in this study, although the study may help pick up symptoms or problems you are experiencing early and help connect you to further care if this is required. Your contribution may help advance our knowledge about COVID19 and its consequences.

Rights as research participant and complaining channels

You may contact the office of Prof Marc Blockman, Chairperson of the UCT Human Research Ethics Committee, at 021 406 6492 with any concerns or complaints that have not been adequately addressed by the research staff.

Who will have access to your medical records?

All information obtained during this study, including your hospital records, samples taken from you, as well as information from the questionnaires, will be kept strictly confidential. When we process the data, we use anonymized profiles where results from samples taken when you were ill, and results of questionnaires are linked with numbers and can only be accessed with passwords. In this process, we remove all the original names and any personal information to ensure that you cannot be identified.

Will you be paid to take part in this study and are there any costs involved?

You will not be paid to participate in the telephonic interviews in this study. A call will be scheduled at a time convenient for you when the researcher will contact you at their expense. You do not have to travel. Participation is entirely voluntary and will not affect your participation in the original parent study (UCT Biorepository). You may withdraw from the study at any time.

Agree / Declined

Name:

……

……..

Contact:

……
…….

Telephonic Interviewer to Tick Boxes.

1. The purpose of the study has been explained to me and I understand what my responsibilities will be if I choose to take part.	
2. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
3. I understand that if I decide at any time that I no longer wish to take part in this project, I can notify the researchers involved and withdraw immediately.	
4. I consent to the processing of my personal information for the purposes of this research study and that such information will be treated as strictly confidential.	
5. I understand that this study is a sub-study of the UCT FHS COVID-19 Biorepository in which I have taken part, and that the original consent applies, that samples taken at the time of and after my illness can be used for this research, and that samples can be stored for future related research.	

6. I agree that the research project named above has been explained to me to my satisfaction and I agree to take part in this study.	
7. I agree to be contacted by the study team by e-mail or by phone during the length of the study and in the future in relation to affiliated studies.	

_____	_____	_____
Name & Surname of Study Participant	**Date**	**Contact**
_____	_____	_____
Name & Surname of Person taking consent	**Date**	**Signature**
_____	_____	_____
Name of Witness to telephonic consent	**Date**	**Signature**

Appendix B: Suicidal Patients

In the event that a participant expresses suicidal ideation, intent, or plans, a risk assessment will be performed by the Research Assistant. This will entail performing the Columbia-Suicide Severity Rating Scale (C-SSRS).

As recommended by the developers of this instrument, namely researcher at the University of Columbia in collaboration with the NIMH, any "Yes" answer on the screen warrants seeking mental healthcare. Participants identified to be low risk will be given a referral list on how to access services.

Participants answering "Yes" to questions 4, 5, or 6 will be identified as high risk and will be contacted by Dr Inette van Niekerk immediately in order to assess, using her clinical judgment and the results of the C-SSRS, whether urgent or emergency care is required.

In the event of imminent risk, the participant will be counselled telephonically by Dr Inette van Niekerk and the patient handed over to the on-site medical practitioner at the most appropriate psychiatric referral facility for emergency assessment and management.

All incidents of high risk suicidality must be documented by the research assistants and contact details recorded for follow-up.

Appendix C: Letter to Participant and Referral Letter

Date: _____

Dear _____

Thank you very much for participating in this study about persistent symptoms experienced by patients post Covid19 infection. From our assessment, it appears that you are experiencing challenges and/or symptoms affecting your mental health.

You may benefit from further assessment and treatment to assist you in overcoming these challenges.

We recommend that you make an appointment at either

- your local Community Health Centre / Community Health Clinic for Mental Health Services, or
- with a Private Practitioner of your choice, such as a Psychiatrist or a Psychologist. You may also choose to see a General Practitioner (GP) who will be able to refer you for more specialized care if necessary.

Please note if you screened positive for high suicide risk, we would be required to facilitate an emergency assessment.

You may also find the following information and emergency contact details helpful:

South African Depression and Anxiety Group (SADAG) Suicide crisis helpline 0800 567 567

SADAG Counselling queries: zane@sadag.org / 011 234 4837 / CIPLA Whatsapp Chat Line 076 882 2775 / SADAG 24-h Mental Health Helpline 0800 456 789

Thank you for participating in this study. Please feel free to contact me if you have any questions or require any further information.

Kind regards

Dr Inette van Niekerk
Psychiatry Registrar; MBBCH (Wits) DMH (SA)
MP0848301
Inette.vanniekerk@gmail.com or 072 399 5960

Date: _____

Medical Practitioner: Mental Health Services

Dear Colleague

RE: Name:_____
DOB:_____
Contact number:_____

Thank you very much for seeing the above patient. During a study at the University of Cape Town investigating persistent symptoms experienced by patients post-Covid infection, it

emerged that this patient is having challenges that would require further assessment and management. Patients were screened for persistent fatigue and anxiety, and also for suicidality. This patient has been referred to you for

- Emergency Assessment and Management
- Non-Emergency treatment.

Suicide risk has been assessed as low / medium / high.
Further information about this patient that may be helpful to you:

_____
_____
_____
_____
_____

Please would you kindly assist this patient with accessing the care that they require.
Please feel free to contact me if you have any questions or require any further information.
Kind regards
Dr Inette van Niekerk
Psychiatry Registrar
MBBCH (Wits) DMH (SA)
MP0848301
Inette.vanniekerk@gmail.com or 072 399 5960

Appendix D: Ethics Approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45, E-52- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

18 January 2022

HREC REF: 711/2021

A/Prof J Peter
Division of Allergology
UCT Lung Institute
Email: jonny.peter@uct.ac.za
Student: Inette2@yahoo.com

Dear A/Prof Peter

PROJECT TITLE: POST-COVID19 ANXIETY AND FATIGUE IN HOSPITALIZED SOUTH AFRICAN PATIENTS AND ASSOCIATIONS WITH ALTERED BRADYKININ PHYSIOLOGY (SUB-STUDY R021/2020 –UCT FHSCOVID-19 BIOREPOSITORY & 257/2020 –ACE2 STUDY) (MMED DEGREE - DR INETTE VAN NIEKERK)

Thank you for your response letter, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020: 06 July 2020 & 01 July 2021.

Approval is granted for one year until the 30 January 2023

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: -Dr Inette van Niekerk will also be involved in this study.

Please quote the HREC REF 711/2021 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Form FHS006: Protocol Amendment

HREC office use only (FWA00001637; IRB00001938)		
☑ Approved	☐ Type of review: Expedited	☐ Full committee
This serves as notification that all changes and documentation described below are approved.		
Signature HREC Chairperson / Designee	Signed by candidate	Date 28/4/2022

Note: All **Major** amendments must include a **Cover Letter** and a local **PI Synopsis** justifying the changes for the amendment. Please note that incomplete amendment submissions will not be reviewed.

Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za with subject line: FHS006 + (HREC Reference number).

The latest forms are found on our website.

http://www.health.uct.ac.za/fhs/research/humanethics/forms

Please also clarify your plan for research-related activities during COVID-19 lockdown.

Comments from the HREC to the Principal Investigator:
Note: The approval of this protocol amendment does not grant annual approval. Please complete the FHS016 / FHS017 form for annual approval at least one month before study expiration.

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	19 April 2022		
HREC REF Number	711/2021		
Protocol Title	Post-Covid19 anxiety and fatigue in South African patients and associations with altered bradykinin physiology (Sub-study R021/2020 UCT FHS COVID-19 Biorepository & 257/2020 ACE2 study) (MMed Degree – Dr Inette van Niekerk)		
Protocol Number (if applicable)	N/A		
Principal Investigator	Associate Professor Jonathan Peter		
Department / Office Internal Mail Address	Division of Allergy & Clinical Immunology, H Floor, Groote Schuur old Main Building, Observatory, Cape Town		
1.1 Is this a major or a minor amendment? (see FHS006hlp) Major (tick box) Minor (tick box)		☐ Major	✓ Minor

Appendix E: Research access to Students

RESEARCH ACCESS TO STUDENTS — DSA 100

NOTES

1. This form must be **FULLY** completed by all applicants who want to access UCT students for the purpose of research or surveys.
2. Return the fully completed **(a) DSA 100** application form **by email**, in **the same word format**, together with **your: (b) research proposal inclusive of your survey, (c) copy of your ethics approval letter / proof (d) informed consent letter** to: Nadierah.Pienaar@uct.ac.za. You application will be attended to by the Executive Director, Department of Student Affairs (DSA), UCT.
3. The turnaround time for a reply is **approximately 10 working days**.
4. NB: It is the responsibility of the researcher/s to apply for and to obtain **ethics approval and to comply with amendments that may be requested;** as well as to **obtain** approval to access UCT staff and/or UCT students, from the following, at UCT, respectively: (a) **Ethics**: Chairperson, Faculty Research Ethics Committee' (FREC) for ethics approval, (b) **Staff access:** Executive Director: HR for approval to access UCT staff, and (c) **Student access:** Executive Director: Student Affairs for approval to access UCT students.
5. **Note:** UCT Senate Research Protocols requires compliance to the above, **even if prior approval has been obtained from any other institution/agency. UCT's research protocol requirements applies to *all* persons, institutions and agencies from UCT and external to UCT who want to conduct research on human subjects for academic, marketing or service related reasons at UCT.**
6. **Should approval be granted to access UCT students for this research study, such approval is effective for a period of one year from the date of approval (as stated in Section D of this form), and the approval expires automatically on the last day.**
7. **The approving authority reserves the right to revoke an approval based on reasonable grounds and/or new information.**

SECTION A: RESEARCH APPLICANT/S DETAILS

Position	Staff / Student No	Title and Name	Contact Details (Email / Cell / land line)
A.1 Student Number	SWRINE001	Dr Inette van Niekerk	Inette.vanniekerk@gmail.com / 072 399 5960 SWRINE001@myuct.ac.za
A.2 Academic / PASS Staff No.			
A.3 Visitor/ Researcher ID No.			
A.4 University at which a student or employee	University of Cape Town	Address if *not* UCT:	
A.5 Faculty/ Department/School	Faculty of Health Sciences		
A.6 APPLICANTS DETAILS If different from above	Title and Name	Tel.	Email

SECTION B: RESEARCHER/S SUPERVISOR/S DETAILS

Position	Title and Name	Tel.	Email
B.1 Supervisor	A/Prof Jonny Peter	021 406 6889/7	Jonny.Peter@uct.ac.za
B.2 Co-Supervisor/s	Prof Dan Stein	021 406 6566	Dan.Stein@uct.ac.za

SECTION C: APPLICANT'S RESEARCH STUDY FIELD AND APPROVAL STATUS

C.1 Degree – if applicable	MMed in Psychiatry
C.2 Research Project Title	Post-Covid19 anxiety, fatigue and cognitive symptoms in South African patients and associations with altered bradykinin physiology
C.3 Research Proposal	Attached: Yes ■ No ☐
C.4 Target population	Registered UCT student cohort of patients already enrolled in ACE 2 (HREC R021/2020) and asymptomatic Covid positive patients from the BCG vaccine study (HREC 237/2020)
C.5 Lead Researcher details	If different from applicant:
C6. Will use research assistant/s	Yes ■ No ☐ If yes- provide a list of names, contact details : PNRMON003, Ms Monica Panieri. PNRMON003@myuct.ac.za, T0078018 Ms Wisahl Wallace, wisahl.wallace@uct.ac.za,
C.7 Research Methodology and Informed consent	Research methodology: Quantitative telephonic questionnaire Informed consent: Yes, prior consent provided for parent study, namely ACE-2 (HREC R021/2020) and BCG vaccine studies (HREC 237/2020)
C.8 Ethics clearance status from UCT's Faculty Ethics in Research Committee /Chair (EiRC)	Approved by the UCT EiRC: Yes ■ With amendments: Yes ■ No ☐ (a) Attach copy of your UCT ethics approval. Attached: Yes ■ No ☐ (b) State date / Ref. No / Faculty of your UCT ethics approval: 28/04/2022 Renewed Ref. / Faculty: 711/2021

SECTION D: APPLICANT/S APPROVAL STATUS FOR ACCESS TO STUDENTS FOR RESEARCH PURPOSE
(To be completed by the ED, DSA or NOMINEE)

D.1 APPROVAL STATUS	Approved / With Terms / Not (i) Approved ■ ~~(ii) With terms~~ ~~(iii) Not approved~~	* Conditional approval with terms a) Access to students for this research study must only be undertaken after written ethics approval has been obtained. b) In event any ethics conditions are attached, these must be complied with before access to students.		Applicant/s Ref. No.: SWRINE001 / Dr Inette van Niekerk
D.2 PREPARED BY:	Designation	Name	Signature	Date of Approval
	Personal Assistant	*Nadierah Pienaar*	Signed by candidate	8/08/2022
D.3 APPROVED BY:	Designation	Name	Signature	Date of Approval
	Executive Director / Nominee Department of Student Affairs	*Mr Pura Mgolombane*	Signed by candidate	*8/08/2022*

Appendix F: Journal Instructions to Authors for *Brain, Behavior, and Immunity*

Your Paper Your Way (available at https://www.sciencedirect.com/journal/brain-behavior-and-immunity/publish/guide-for-authors)

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.

To find out more, please visit the Preparation section below.

Types of Article

Original full-length research reports, full-length review articles,short communications, brief commentaries, and letters to the editor will be considered for publication.

Full-length research reports: The chief criteria for the acceptance of submitted papers are the quality, originality, and clarity of the work reported, addressing one or more of the research areas reported above. There is no word limit on full length research reports, but papers should be consisely written and most should be able to articulate their findings within approximately 6,000 words.

Preparation

Queries

For questions about the editorial process (including the status of manuscripts under review) or for technical support on submissions, please visit our Support Center.

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality Figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual Figure files larger than 10 MB must be uploaded separately.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged.

The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions. If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes. Divide the article into clearly defined sections.

Figures and tables embedded in text

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.