Brain, Behavior, & Immunity - Health 46 (2025) 100990

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

# Acute serum protein biomarker profile and prevalence of persistent (>6 months) neuropsychiatric symptoms in a cohort of SARS-CoV-2 PCR positive patients in Cape Town, South Africa

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ARTICLE INFO

*Keywords:*
Anxiety
Cognitive impairment
Fatigue
Immunopathological
Long-term neuropsychiatric sequelae
Memory
Neuropsychiatric
Post-acute sequelae of COVID-19
Post COVID-19
SARS-CoV-2
Renin-angiotensin system
Serum immunoproteome

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 African cohorts and few acute illness biomarkers of persistent neuropsychiatric symptoms.

*Objectives:* To examine the association of neuropsychiatric outcomes with clinical illness severity, systemic inflammation, cardiovascular and renin-angiotensin-system (RAS) biomarkers. Second, 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.

*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. The 96-protein O-link inflammation and cardiovascular panels, RAS fingerprinting, and antibody responses were measured in serum samples collected at peak severity and recovery (>3 months post-infection). 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).

*Results:* Ninety-seven participants completed telephonic interviews. The median (IQR) age was 48 (37–59) years, and 54 % were female. There were no significant associations between neuropsychiatric outcomes and illness severity, systemic inflammation, cardiovascular and/or renin-angiotensin-system (RAS) biomarkers from either peak illness or recovery samples. More than half of this SA COVID-19 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.

*Conclusion:* The high prevalence of persistent neuropsychiatric symptoms in this African cohort supports ongoing attention to long COVID. Acute and early serum protein biomarkers were not associated with persistent neuropsychiatric outcomes post-COVD-19.

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https://doi.org/10.1016/j.bbih.2025.100990
Received 11 June 2024; Received in revised form 3 March 2025; Accepted 3 April 2025
Available online 16 April 2025

**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 |
| NIAID | National Institute of Allergy and Infectious Diseases |
| NIH | National Institutes of Health |
| NMDA | N-methyl-D-aspartate |
| NPX | Normalized Protein expression Scores |
| PASC | Post-Acute Sequelae of COVID-19 |
| PCA | Principle Component Analysis |
| PCR | Polymerase Chain Reaction |
| POPIA | Protection of Personal Information Act |
| 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 |
| Tele-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 |

## 1. Introduction and background

The COVID-19 pandemic has significantly impacted the health of populations around the world. South Africa saw a total of 4.07 million confirmed cases and >102 595 deaths (World Health Organization). SARS-CoV-2 has the potential to cause multisystemic complications, including neurological and neuropsychiatric manifestations, and also ongoing symptoms well beyond the acute infection (Huang et al., 2021; Nalbandian et al., 2021). The World Health Organisation's (Soriano et al., 2022) standardised clinical case definition of the long COVID-19 condition (also known as post COVID-19 or post-acute sequelae of COVID-19 [PASC]) (Soriano et al., 2022) 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 (Soriano et al., 2022). The definition makes provision for new onset as well as persistent symptoms, and no minimum number of symptoms are required for the diagnosis (Soriano et al., 2022).

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 COVID-19 survivors experienced at least one unresolved symptom (mean follow-up 126 days), with fatigue being the most prevalent (Mahone et al., 2023). A recent systematic review (Müller et al., 2023) on Long COVID on Africa found that prevalence rates varied from 2 % in Ghana, to 86 % in Egypt. The prevalence of long COVID in the four South African studies ranged from 24 to 67 % (Dryden et al., 2022a; Mendelsohn et al., 2022; Pretorius et al., 2022; Wose Kinge et al., 2022). This review found that the most common reported symptoms in Africa were also neuropsychiatric in nature.

In a study of COVID-related neuropsychiatric sequelae using large databases of Electronic Health Records from the US, 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 (Taquet et al., 2021, 2022). 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 (Taquet et al., 2022). A comprehensive PASC symptom lexicon compiled from EHRs found that anxiety (25.8 %), depression (24.0 %), and fatigue (23.4 %) were particularly prevalent (Wang et al., 2022).

There are currently no biomarkers in clinical practice able to predict long COVID-19. Recent systematic reviews suggest both inflammatory (IL-6, CRP and TNF-α) and neuron-specific biomarkers (neurofilament light chain (NFL) and glial fibrillary acidic protein) are increased in long COVID-19 compared to both recovered or healthy control patients (Yun-Ju et al., 2023). Different biomarkers have been linked with different long COVID-19 outcomes, with persistent elevation of chemokine CCL11 associated with cognitive dysfunction (Davis et al., 2023). Some have examined the possibility that cardiovascular markers, and specifically derangements in the RAS and kallikrein-kinin system, can predict long-COVID (Cojocaru et al., 2023). Our updated literature review identified several recent studies examining for serum proteomic markers associated with long-COVID19; however all studies were from cohorts in HICs and few with the combination of sampling on acute COVID19 hospitalization matched to longer-term neurocognitive outcomes (>6months) (see Supplementary Table S7) (Cojocaru et al., 2023).

While some data is available on long-COVID from LMICs (and in particular from Africa), Taghrir et al. still found that most studies on long COVID came from high and upper-middle income countries (Taghrir et al., 2022). This results in neglecting some vulnerable populations where services for long COVID are more likely limited (Taghrir et al., 2022). 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 (McDonald, 2017). In South Africa, Dryden et al. investigated long COVID-19 at three months post-discharge, with two thirds of hospitalized COVID-19 patients reporting new or persistent COVID-19-related symptoms (Dryden et al., 2022b), 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 (Jassat et al., 2023). However, little is known regarding outcomes after 6-months.

Our study aimed to address several of these important research areas. First, by determining the prevalence of persistent neuropsychiatric symptoms (>6 months post-infection/hospitalization) in a cohort of South African SARS-CoV-2 PCR positive patients across the COVID-19 disease severity spectrum. Second, to determine if demographic, clinical, and peripheral blood markers including systemic inflammatory, cardiovascular disease, RAS fingerprinting and antibody response was associated with these persistent neuropsychiatric outcomes.

## 2. Methods

### 2.1. Study setting, participants, and severity classification

A random selection of patients admitted with COVID-19 (SARS-CoV-2 PCR positive) to Groote Schuur Hospital (GSH) were included as part of the UCT FHS COVID-19 biorepository and ACE2 study enrolments. The consort diagram indicates drop-outs vs retention from the original cohort to those participants undergoing telephonic interviews. Please see sensitivity analysis (Table S5 A &B in Supplementary materials) for comparison of characteristics of included participants versus those who were not included in the study. Admissions were from June 21, 2020 until August 3, 2021, across the first three waves of COVID-19 infections in South Africa (dominant variants: Ancestral, Beta and Delta) (Suliman and Mtsweni, 2022). 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 vaccination (<7 days), meaning it is reasonable to still consider vaccine-naïve. 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, to ensure 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 discovered that these patients have had seroconverted (anti-N IgG) indicating recent 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 (World Health Organization; Huang et al., 2021; Nalbandian et al., 2021; Soriano et al., 2022; Mahone et al., 2023; Müller et al., 2023; Dryden et al., 2022a; Mendelsohn et al., 2022; Pretorius et al., 2022) per patient. Details of GSH and the drainage population, and geographical area served by the hospital, are provided in the supplementary materials. A group of asymptomatic, non-hospitalized COVID-19 participants from the same population, namely residing in the area of Cape Town serviced by GSH, were also included from the BCG Study, full details of which are published (Caryn et al., 2022). Patients entering the BCG study had blood panels drawn, and those found to be COVID wild-type antibody positive with no history of clinical COVID disease were approached for inclusion in the Post-COVID study. Telephonic contact and recovery sampling, wherever possible, were attempted for all discharged patients 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 COVID-19 disease severity classification (World Health Organization, 2020), 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 (National Institutes of Health: COVID-19 Treatment Guidelines Panel, 2020) (US Food and Drug Administration, 2020). 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 (Beigel et al., 2020): 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 ≤ 300 and > 100); NIAID 6–7 (hospitalized with WHO critical disease or PaO2/FiO2 ≤ 100). The detailed methodology for severity classification is available in the supplement (Tables 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. Routine and specialised laboratory data and methods

Participants enrolled in the context of the acute UCT FHS COVID-19 biorepository did not have protocol specified blood sampling or laboratory testing; however standard-of-care at GSH for COVID-19 in-patients (Mendelson et al., 2020) 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, these analytes 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 (Reindl-Schwaighofer et al., 2022).

Anti-nucleocapsid and anti-spike IgG measurements, as well as RAS fingerprinting were performed for one 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]). Not all participants had O-link panels done because recovery blood samples could not be obtained from all participants.

There were some missing data for some of the participants. Table S6 in the supplementary materials describe the number of O-link samples obtained and analysed in each severity category from the post-COVID cohort.

Further detailed laboratory methods are provided in the supplementary materials.

### 2.3. Telephonic assessments of neuropsychiatric symptoms

All eligible participants were contacted and consented to telephonic assessment for persistent neuropsychiatric symptoms and completion of validated instruments. 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 South African cohort with very low rates of pre-morbid psychiatric illness or cognitive impairment. Once-off telephonic interviews were done middle 2022 for participants infected across all 3 COVID waves; between 6 months and 2 years post infection. We explored whether renin-angiotensin system (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.

Telephonic interviews and questionnaires were administered by trained healthcare workers. 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 Tele-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). The GAD-7 and CFS-11 have been recommended for the study of patients with COVID-19 (Cysique et al., 2021), and the SRQ-20 and GAD-7 have been validated for use in LMICs (Mughal et al., 2020). 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**. Information on validation, accuracy and cut-off points of instruments are included in the Supplementary methods.

### 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. This paper reports the prevalence of neuropsychiatric symptoms at >6-month follow-up from acute COVID-19. 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 on 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, open ware R (STATA 15.1 Rv and Team R, 2020) (The R Development Core Team, 2018) and Microsoft excel (version 16.77, Redmond, Washington) (Microsoft Corporation, 2018). 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. 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.

#### 2.4.1. 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 S6). 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 visualized 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-value <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 COVID-19 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 Fig. 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). The sensitivity analysis in Table S5 A&B in the Supplementary materials show that 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.

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 hospitalized cohort was 157 (74–270), indicative of the profound hypoxemia in hospitalized COVID-19 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. Inflammatory, cardiovascular, 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) interviewed participants, respectively. The peak illness severity for sampling was 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 S6).

The analyses presented in the Heatmaps and Principle Component Analysis plots and the supplementary materials include biomarkers of RAS (Fig. 3a–d), antibody responses (Fig. 3e–h), Olink inflammatory and cardiovascular disease (Fig. 2a and b). Using neither samples taken at peak illness severity (Fig. 2a and b) nor recovery (see Supplementary materials Figure S8B), could principal component analysis of proteins using normalized protein expression (NPX) levels or unsupervised hierarchical cluster analysis differentiate between affected and unaffected individuals for any of the persistent neuropsychiatric outcome; in contrast to the clear pattern of clustering associated with different

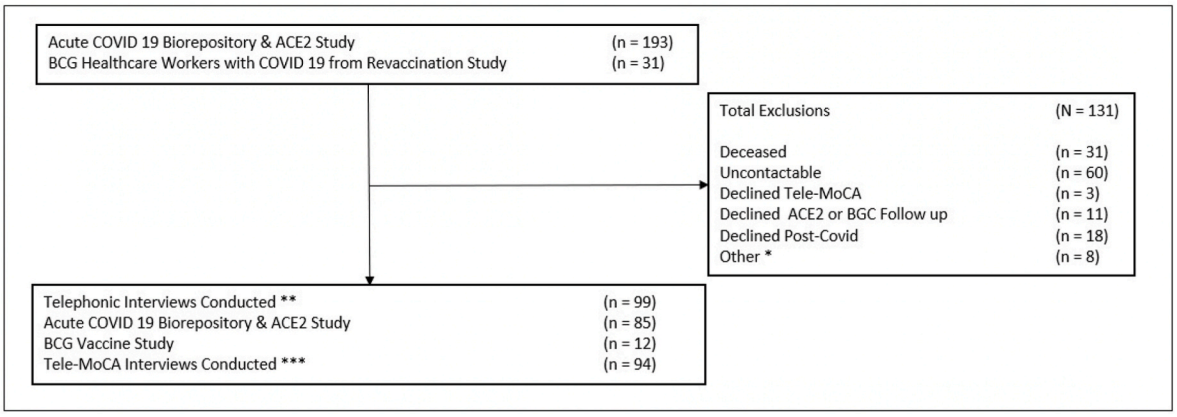


**Fig. 1. Consort diagram – total final participants n = 97; total final Tele-MoCA n = 93**

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

ACE2 = Angiotensin-converting enzyme 2

BCG = Bacillus Calmette-Guerin

Tele-MoCA = Telephonic Montreal Cognitive Assessment.

**Table 1**

Baseline characteristics of interviewed patients stratified by neuropsychiatric outcomes.

| Characteristics | Complete Cohort, n = 97 | T-MoCA* | | Subj** | | GAD*** | | Fatigue**** | |
|---|---|---|---|---|---|---|---|---|---|
| | | Unimpaired, n = 52 | Impaired, n = 41 | Unreported, n = 46 | Reported, n = 51 | Negative, n = 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 (16) | 10 (19) | 6 (31) | 8 (17) | 8 (16) | 12 (16) | 4 (17) | 8 (19) | 8 (31) |
| - Myalgia | 6 (6) | 5 (10) | 1 (34) | 1 (34) | 5 (10) | 4 (5) | 2 (8) | 2 (5) | 4 (7) |
| - Headache | 6 (6) | 4 (8) | 2 (5) | 3 (7) | 3 (6) | 2 (34)***** | 4 (17)***** | 2 (5) | 4 (7) |
| - GIT symptoms | 6 (6) | 3 (6) | 3 (7) | 4 (9) | 2 (31) | 5 (7) | 1 (31) | 4 (9) | 2 (31) |
| 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 (5) | 2 (31) | 2 (6) | 2 (5) | 2 (31) | 1 (34) | 3 (14) | 2 (5) | 2 (31) |
| 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 (35) | 13 (32) | 11 (24) | 16 (31) | 21 (29) | 6 (35) | 12 (28) | 15 (28) |
| HIV, x (%) | 4 (31) | 2 (31) | 2 (5) | 1 (34) | 3 (6) | 3 (31) | 1 (31) | 0 (34) | 4 (7) |
| 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)****** | 122 (77–169)****** | 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) | 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 *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 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.

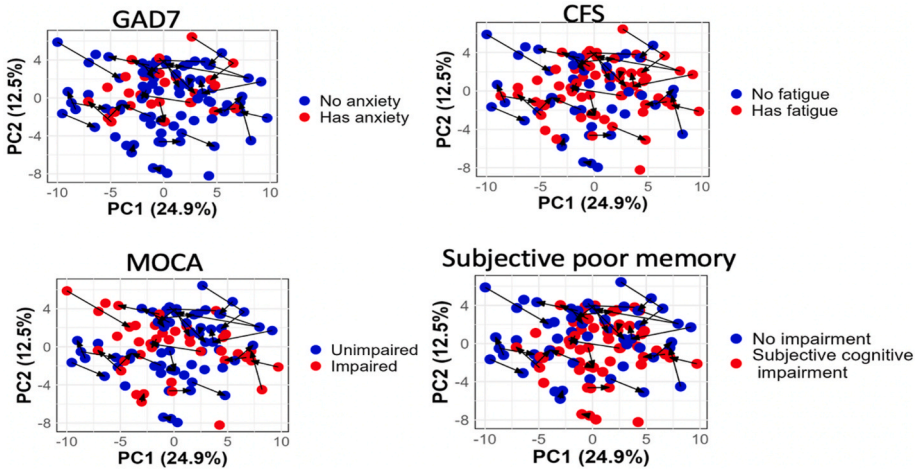


**Fig. 2a. O-link inflammatory panel PCA plot at peak illness severity**

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 Tele-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.

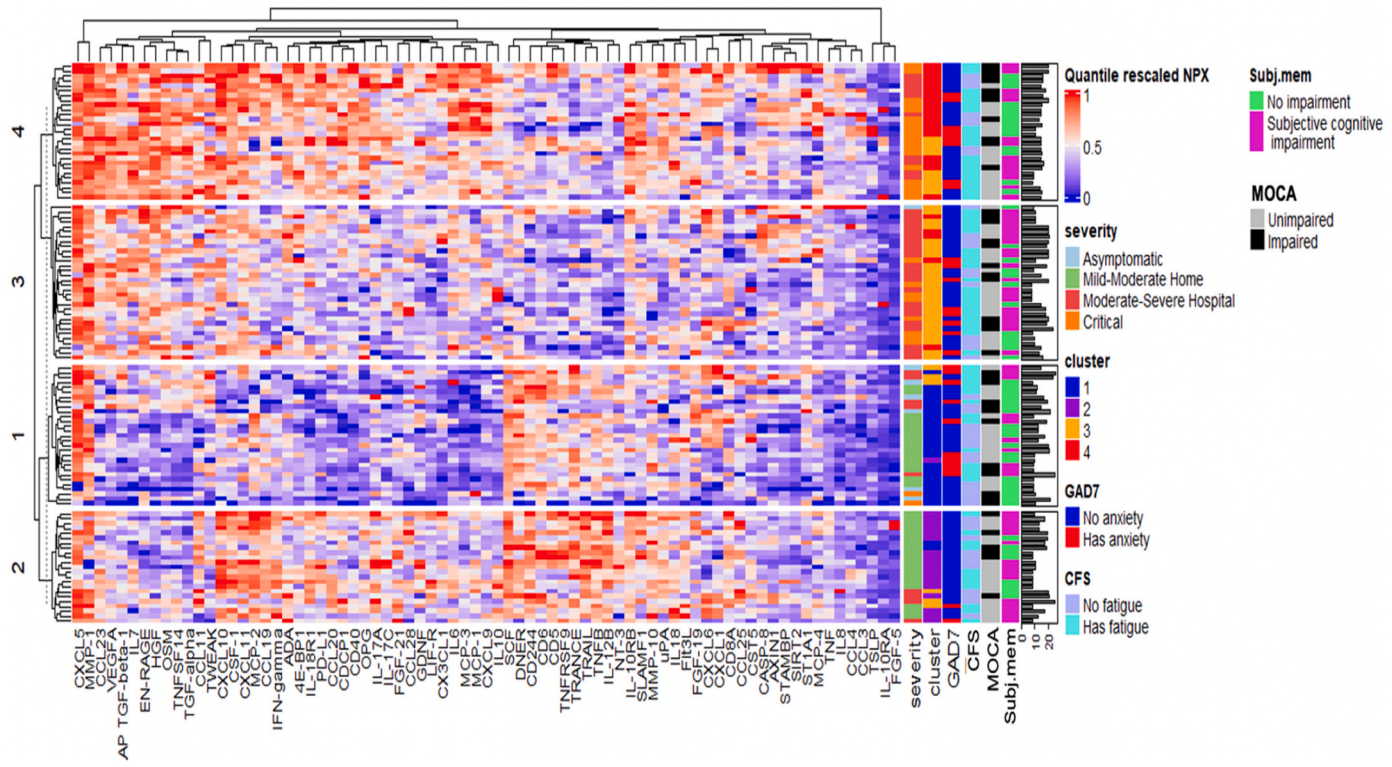


**Fig. 2b. O-link inflammation Heatmap at Peak**

* 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.

disease severity groups. 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 (Supplementary Figure S8A).

### 3.3. Neuropsychiatric outcomes

Fig. 4a–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 COVID-19 infection, which increased to 42/97 (43 %) post COVID-19 infection (the original 25 and additional new 17) (Fig. 4a and b);

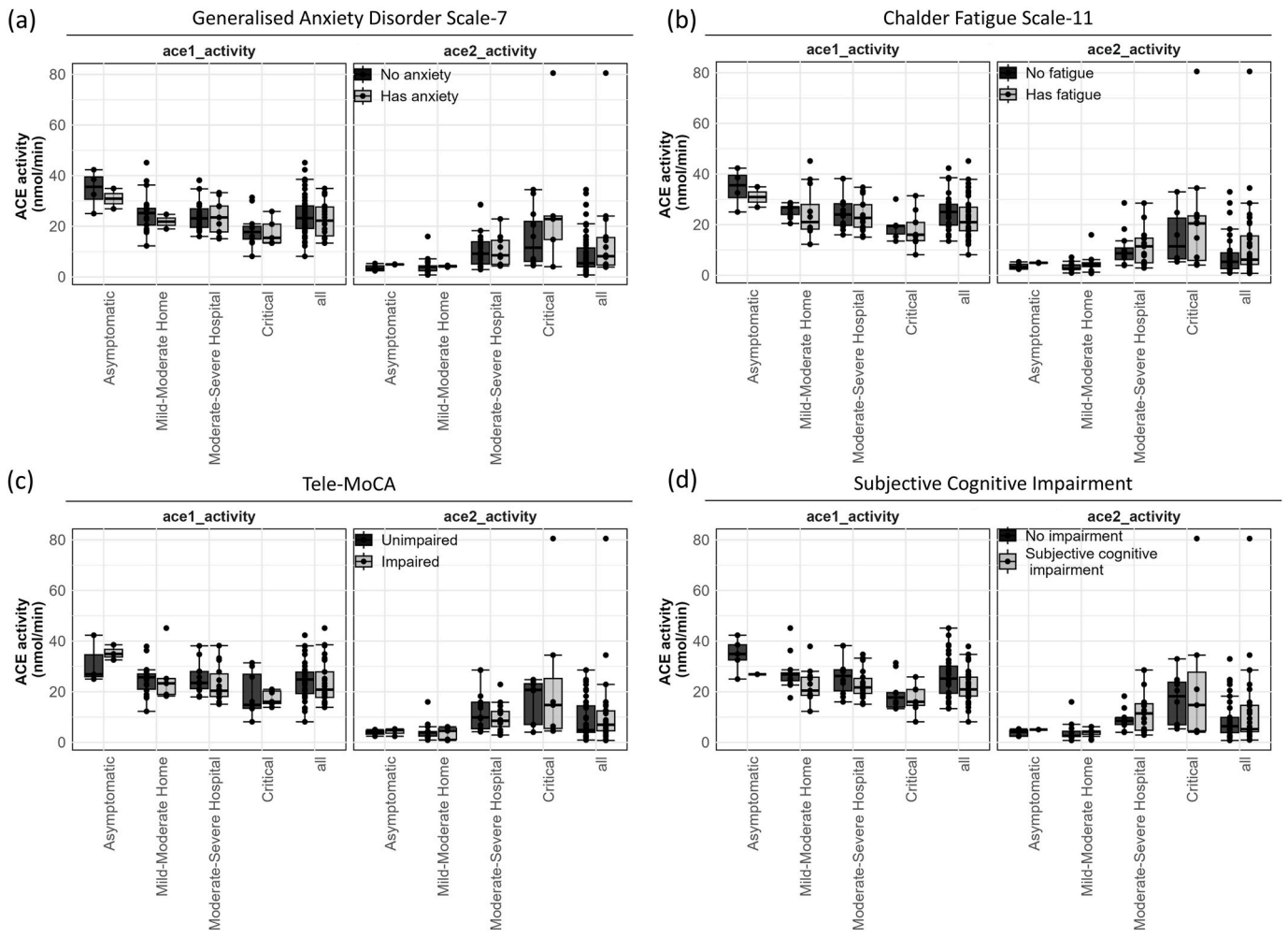


**Fig. 3a–d. ACE and ACE-2 activity across severity groups in different neuropsychiatric outcomes**

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 Tele-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.

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 (Fig. 4c), 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) versus14 %, (6/42) $p = 0.03$]. Levels of fatigue measured at the various time points after participants had COVID-19, as per the CFS-11 with cut-off of 4 or more (47) (Fig. 4d), 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-values = 0.0535) and 11/20 (55 %) in moderate-severe hospital and critical disease severity groups (p-value = 0.0269). Sixty-two 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 COVID-19 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 percent of participants reported word finding difficulties on the CFS-11 and case report forms (Fig. 3h). In many instances, participants also reported that their family members commented on this post-discharge. Phonemic fluency, an objective T-MoCA outcome measure (Fig. 3g), was below the 11-word normal threshold in 52/93 (56 %) of participants. There were no 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, 24/93 (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

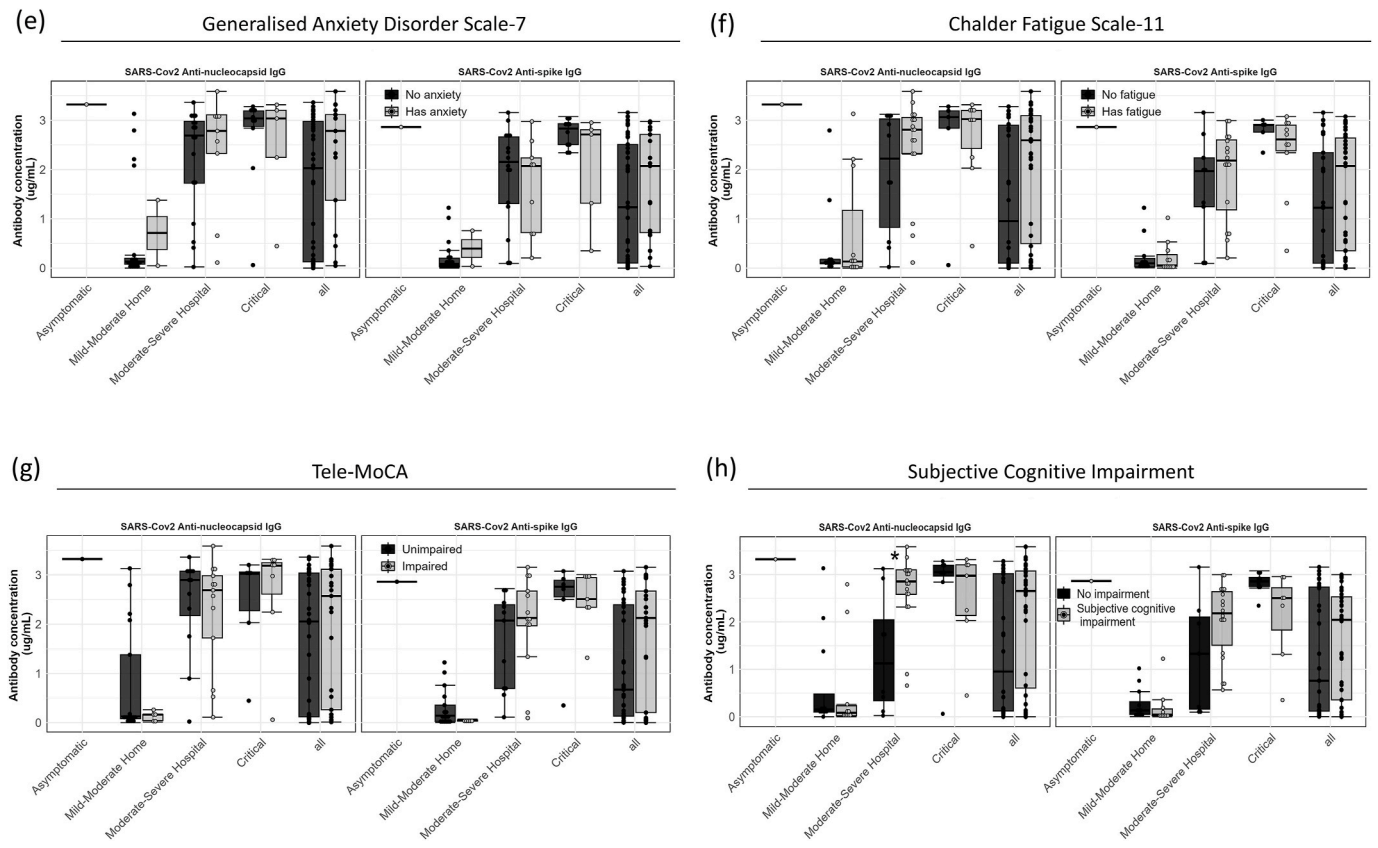


**Fig. 3e–h. Antibody responses to SARS-CoV-2**

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 Tele-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.

initially could not remember any words, were able to remember additional words on category or multiple choice cue.

### *3.4. Telephonic interview timing post COVID-19 infection and neuropsychiatric outcomes*

Patients in this cohort were recruited across three different COVID-19 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 COVID-19 infection. Fig. 5A-D shows neuropsychiatric outcomes plotted against the time since SARS-CoV-2 PCR positivity, stratified by illness severity category. This figure illustrates that 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.

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 (Fig. 5A-D). The bold black line depicts overall neuropsychiatric outcome, not stratified be either anxiety, fatigue, objective and subjective cognitive impairment. There were no significant differences depicted in the distribution by wave (see Supplement Table S4).

## 4. Discussion

Our study explored whether renin-angiotensin system (RAS) and 96-marker plasma inflammatory markers or cardiovascular proteomic markers measured during peak illness severity or a recovery time-point (>3 months from COVID19) were associated with persistent neuropsychiatric outcomes. Examining for early biomarkers has been a focus of intense study given the high reported rates of persistence of neuropsychiatric symptoms after acute COVID19 illness, however few studies have managed to collect early admission sampling, conduct complex measurements such as RAS, and study longer term neurocognitive COVID19 outcomes. Indeed, in this study of a South African post COVID-19 cohort with a median age of 48 years, more than half of participants were affected by neuropsychiatric symptoms of anxiety, fatigue, memory and cognitive impairment, some more than 24 months from their incident illness. This highlights a considerable disease burden.

Supplementary table S7 details published similar studies with acute illness serum (and CSF) proteomic sampling and measurement of long COVID19 neuropsychiatric outcomes (Schmitz et al., 2024; Ozonoff

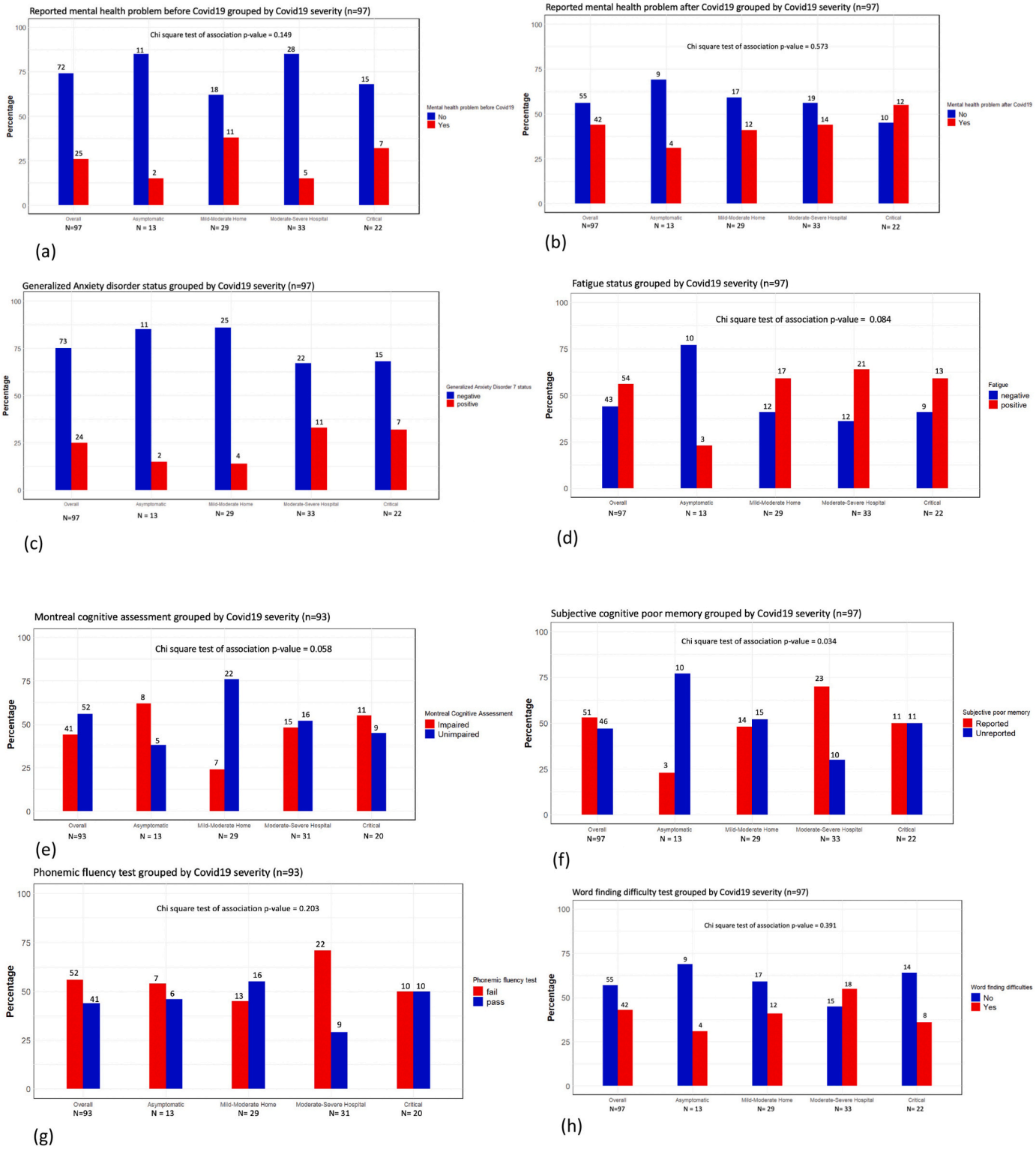


**Fig. 4.** a–h: **Mental health status before and after COVID-19 and Neuropsychiatric outcomes >6 months after infection**
a) Reported mental health problems prior to infection with COVID-19 overall (far left) and in each different COVID-19 severity group
b) Reported mental health problems after infection with COVID-19 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 Tele-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 Tele-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.

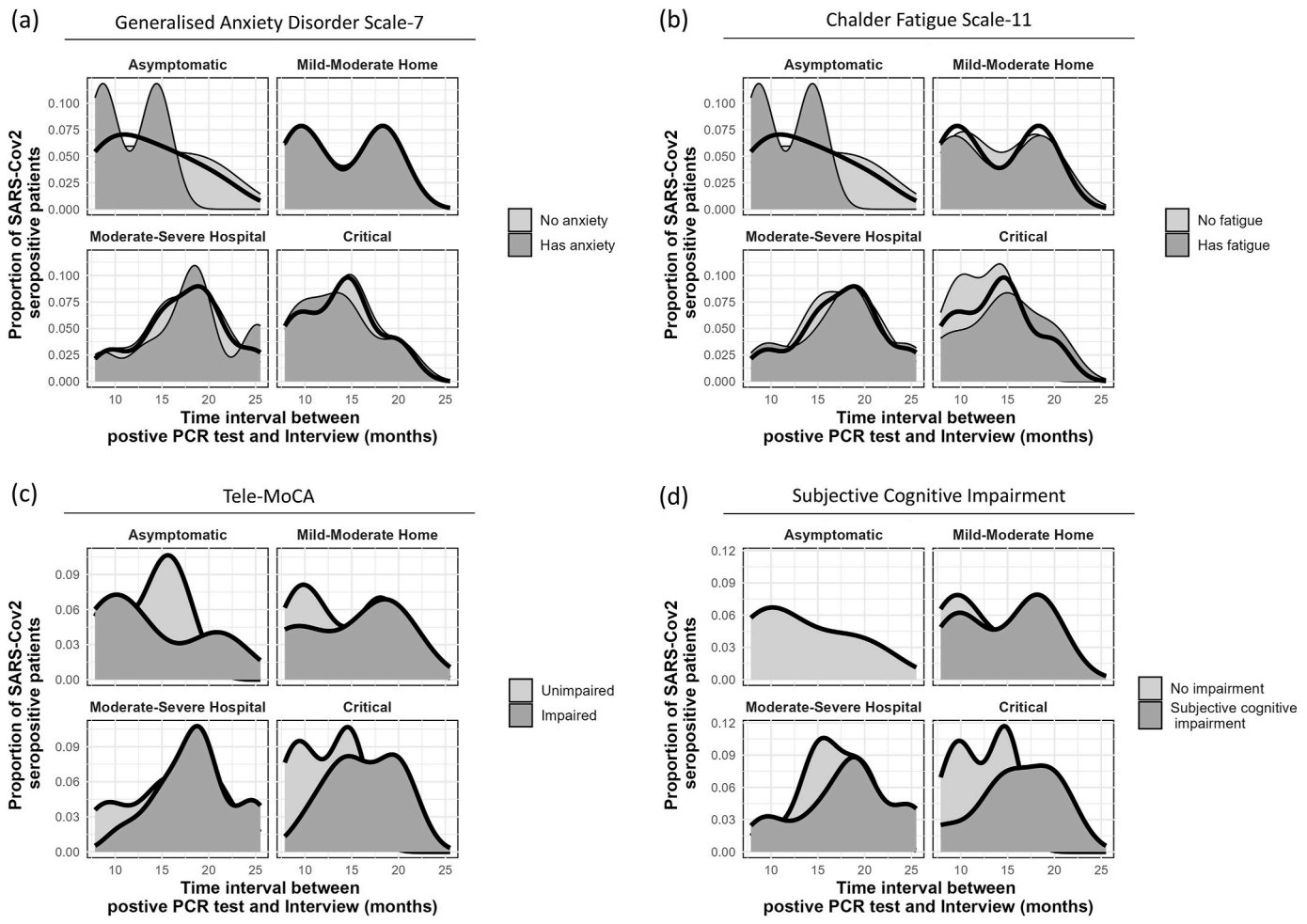


**Fig. 5.** Neuropsychiatric outcomes by time since positive SARS-CoV-2 PCR test stratified by illness severity groups. This figure shows the distribution of study participants (y-axis is the probability density of patients) by time interval between PCR test and interview for A) Generalized anxiety disorder, B) Chadler Fatigue, C) Montreal Cognitive Assessment and D) Subjective Cognitive Assessment. The black solid line shows the overall distribution without the stratification by neurological outcome, and since the graphs of impaired and unimpaired for a particular outcome overlap this indicates that there are no difference based on the time between PCR and interview.

et al., 2024; Lu et al., 2024; De Lorenzo et al., 2024; Patel et al., 2023; Guasp et al., 2022; Bizjak et al., 2022). The absence of any cohorts from LMICs is noteworthy and an important gap which our study addresses. Furthermore, the lack of consistent acute serum biomarkers predictive of longer-term neuropsychiatric sequelae in these existing studies aligns with our results. Despite examining both peak illness and recovery time-point serum samples, there were no differences in ACE activity, antibody responses or plasma inflammatory or cardiovascular o-link markers linked to the presence or absence of neuropsychiatric sequelae. Several possible explanations may account for this finding. First, the intensity, duration, and resolution of neuroinflammatory processes such as microglial activation (Davis et al., 2023) may be distinct from systemic inflammation, and consequently 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 virus or viral persistence (Davis et al., 2023; Low et al., 2023). 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 COVID-19 severity may exist (El-Agnaf et al., 2023). If such mechanism entails autoantibody damage as some have argued (Davis et al., 2023; El-Agnaf et al., 2023), it may not be reflected by serum inflammatory markers. Finally, psychosocial factors could possibly account for this lack of predictive utility that acute illness serum biomarker have for longer term neuropsychitric outcomes, as the impact of the COVID19 pandemic should not be underestimated, and could worsen over time (Thurner and Stengel, 2023; Mahlangu et al., 2022). This explanation is further supported by the inconsistent results seen in other similar studies (Schmitz et al., 2024; Ozonoff et al., 2024; Lu et al., 2024; De Lorenzo et al., 2024; Patel et al., 2023; Guasp et al., 2022; Bizjak et al., 2022). Our work and existing work on long COVID from around Africa show a substantial prevalence of long COVID, with high rates of neuropsychiatric symptoms. This emphasises the need for ongoing COVID prevention measures, including vaccination, to minimise the impact on individuals and society, reduce the potential negative economic effects, and reduce the strain on already taxed healthcare systems. Additionally, this is a call to action to improve diagnosis, reporting of, and access to care for patients in LMIC suffering from long COVID.

The aetiology of psychiatric illnesses is often multifactorial and researchers are now emphasizing the biopsychosocial (cultural-spiritual) approach to the management of long-COVID (Thurner and Stengel, 2023). 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 avoidant behavior and decrease anxiety. Neurocognitive training is advised, and the role of occupational therapists is emphasized to train and/or rehabilitate necessary abilities

thereby enabling work-life participation for affected patients (Thurner and Stengel, 2023). Research findings showed increased risk for domestic violence during lock-down in South Africa specifically affecting those of lower socio-economic status (Mahlangu et al., 2022), in addition to the profound negative economic effects of the pandemic (Mahlangu et al., 2022). Our findings support this integrated care model given the significant long-term morbidity and lack of obvious early biological predictor.

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 South African cohort (median age 48) with very low rates of pre-morbid psychiatric illness or cognitive impairment. 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. The high rates of persistent psychiatric and cognitive sequelae in this South African cohort are noteworthy, and consistent with other cohorts from 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 (Zeng et al., 2023). More specifically, prevalence rates of persistent symptoms among survivors recovered from acute illness were 28.7 % for fatigue (in their study categorised under generalized symptoms); 18.3 % for depression; 16.2 % for anxiety; 19.7 % for cognitive deficits; and 17.5 % for memory impairment (Zeng et al., 2023). 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 (Jassat et al., 2023). 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 (Jassat et al., 2023). 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 (Dryden et al., 2022c). Similar to our findings, persistence of fatigue has been linked to severity of acute illness (Ceban et al., 2022). 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 follow-up period (Taquet et al., 2022; Ceban et al., 2022).

The high rates of self-reported memory and cognitive impairment (which were confirmed on objective testing on the T-MoCA) are also consistent with other work. In the COVCOG study, researchers predicted that participants who had self-reported cognitive symptoms will reflect as such in objective tests (Cysique et al., 2021). With the exception of the asymptomatic group, this was true for our cohort with good correlation between objective and subjective cognitive impairment. The COVCOG study particularly found reduced memory performance on multiple verbal memory tasks (Cysique et al., 2021), 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 (Guo et al., 2022). 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 Tele-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' (Julayanont et al., 2013), clinical features consistent with subcortical white matter damage (Combrinck and Patel, 2022). Microglial activation and microinfarcts are key pathophysiological mechanisms responsible for COVID19-related white matter damage (George, 2022). 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 (Cysique et al., 2021; Hadad et al., 2022); and has been reported to be nearly 70 % in a USA neurology clinic study amongst mild to moderate COVID19 disease severity (Shanley et al., 2022). The median 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 COVID-19 infection suggests these are attributable to COVID-19 infection.

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, education levels and not only COVID19 infection could have affected the Tele-MoCA results in this group (Katz et al., 2021; Rabinovici et al., 2015; Gagnon et al., 2013). 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 reports have all been done at one time-point – participants were asked to reflect back on mental health symptoms and reports 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. Hence our study cannot establish causality. Fourth, 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. Fifth, we did not address psychosocial stress experienced by participants per se. Lastly, due to difficulties experienced in establishing telephonic contact with all participants, follow-up studies are not planned at this stage.

In summary, our findings confirm a high and prolonged impact of acute COVID19 infection on a cohort of South Africans (median age 48) throughout the first three COVID waves. These findings are consistent with other findings in Africa and globally, 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 the future, long COVID care programmes may be incorporated into existing psychiatric care platforms, with particular emphasis on occupational therapy.

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. It is possible that psychosocial effects of COVID19 have influenced symptom expression in this South African cohort. In fact, new hope for psycho-physiological treatments which have shown efficacy, as found by other research teams (Thurner and Stengel, 2023; Cheng et al., 2023; Fine et al., 2022), 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. Psychosocial variables affecting individual responses to the virus may explain the lack of association found on the biological front.

## CRediT authorship contribution statement

**Inette van Niekerk:** Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Monica Panieri:** Project administration, Data curation. **Talitha Müller:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition, Formal analysis, Data curation. **Lovemore Mapahla:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Sonwabile Dzanibe:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Cascia Day:** Writing – review & editing, Project administration, Data curation. **Dan J. Stein:** Writing – review & editing, Supervision, Conceptualization. **Jonny Peter:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

None.

## Acknowledgements

Research reported in this work was supported by the South African Medical Research Council with funds received from the South African Department of Science and Innovation.

CD's thesis is supported by the SAMRC Research Capacity Development, and the Discovery Foundation Academic Award programme.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2025.100990.

## Data availability

Data will be made available on request.

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