COV-N-PSY | COVID-19 NEURO-PSYCHIATRIC CONSEQUENCES

Neurobiological underpinnings of long COVID neuropsychiatric consequences: exploration of two translational pathways

Livia De Picker[1,3], Katrien Skorobogatov[1,3], Filip Van Den Eede[2,3], Kurt Beeckmans[1], Greta Moorkens[2], Bernard Sabbe[3], Manuel Morrens[1,3]

Protocol version 4.0 - Date: FEB2022

[1] University Psychiatric Hospital Duffel
[2] University Hospital Antwerp
[3] Collaborative Antwerp Psychiatric Research Institute, University of Antwerp

Table of Contents

Summary

The coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused more than 120 million cases and 2.6 million deaths to date, since 2020. As the pandemic spread, there has been a growing recognition of neuro-psychiatric (NP) impact of COVID infections. Such complications have been described during the acute phase of the infection with emerging data showing that, particularly in severe cases of COVID-19 infection, the acute viral infection is associated with delirium, confusion, and psychosis, possibly related to the cytokine storm (Rogers et al. 2020). Importantly, a much larger proportion of even mild COVID-19 cases experience neuropsychiatric complications at follow-up. Six months after clinical recovery, an estimated 34% of COVID-19 survivors are diagnosed with new-onset neuropsychiatric disorders, in particular anxiety disorders (17%), mood disorders (14%), substance use disorders (7%) and insomnia (5%) (Taquet et al., 2021). It is clear that long COVID NP symptoms threaten the quality of life and the societal functioning of a significant proportion of the active population (De Lorenzo et al., 2020; Ma et al., 2020; Mazza et al., 2020; Varatharaj et al., 2020, Gennaro et al, 2021). It is estimated that this will severely impact the health of patients infected with COVID-19, long after the pandemic will have ended thanks to vaccination efforts. Despite the major socio-economic consequences of the long-term impact of the pandemic, a standardized way to screen, diagnose, predict and specifically treat patients who develop or will develop these NP symptoms is currently not available. Despite post-viral or post-infectious neuropsychiatric symptomatology has been recognized and studied prior to the COVID-19 pandemic, crucial knowledge about the neurobiological mechanisms underlying these symptoms is still lacking.

Large population cohort studies have replicated the finding of systemic immune disturbances in cohorts of patients with depression. Among post-COVID NP patients, baseline systemic inflammation (systemic immune inflammatory index: SII= thrombocyte count x neutrophile count / lymphocyte count) predicted severity of depressive psychopathology at the three-months follow-up (Mazza et al, 2021).

Also, long COVID can be regarded as a condition of chronic stress, which could theoretically be associated with disturbances of the hypothalamo-pituitary-adrenal (HPA) axis, although no studies on HPA axis (dys)function in long COVID have been reported yet.

The aims of this study are to describe the standardized evaluation of the psychopathological and neurocognitive function of long COVID patients and their evolution over time; to explore the cross-sectional and longitudinal association

between long COVID symptoms and immunological measures; and to examine the neuro-endocrine (HPA-axis) characteristics of long COVID syndrome.

Three participant groups will be included: 1) long COVID patients with neuropsychological complaints (P), 2) COVID-survivors without persistent complaints (Ca) and 3) healthy volunteers (Cb).
This is a longitudinal study, starting from November 1st 2021. Enrollment will continue for an estimated period of 24 months, depending on the time course of the epidemic curve. Last-patient-in is expected no later than November 1st, 2023.
The study will be organized into three work packages (WP1, WP2 and WP3). WP1 includes psychiatric assessments using questionnaires and blood sampling and is the standard WP for every participant. WP2 includes cortisol measurement using saliva sampling for patients and controls from Cb. who do not fulfill specific exclusion criteria (pregnancy, breastfeeding, hormonal therapy with exception of contraceptives, cortisol treatment). Finally, WP3 includes an extensive neurocognitive assessment by a specialized neuropsychologist for both patients and controls from Ca who do not fulfill specific exclusion criteria (sedative medication, pre-existing neurological disease, severe substance abuse, and IQ < 90).
Patients and controls from Ca will be referred mainly by the University Hospital of Antwerp (UZA) and by long COVID patient support groups and enrolled at first visit to UPCD, where follow-up will also take place at 3, 6 and 12 months. WP1 will be performed at each timepoint, while WP2 and WP3 will only be carried out at baseline and 6 months.
Total sample size is estimated on 130 participants.

List of Abbreviations

- ACTH: adrenocorticotropic hormone
- BACS: brief assessment of cognition in schizophrenia
- BDI: Beck depression index
- Ca: Control group a: COVID-survivors without NP complaints
- CAR: cortisol awakening response
- Cb: Control group b: healthy controls
- CFS: chronic fatigue syndrome
- CNS: central nervous system
- COVID-19: coronavirus disease of 2019
- CRF: corticotropin-releasing factor
- CRP: C-reactive protein
- DNA: deoxyribonucleic acid
- DRUID: driving under the influence of drugs, alcohol and medicines
- EDTA: ethylenediaminetetraacetic acid
- FU: follow-up
- HDL: high-density lipoprotein
- HERV-W-ENV: human endogenous retrovirus-W envelope
- HPA: hypothalamic-pituitary axis
- IFN-g: interferon gamma
- IL-1b: interleukin 1-b
- IL-6: interleukin 6
- NESDA: Netherlands Study of Depression and Anxiety
- NP: neuropsychiatric
- PET: positron emission tomography
- P: patient group
- PTSD: post-traumatic stress disorder
- RT-PCR: reverse transcription polymerase chain reaction
- SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
- SII: systemic immune-inflammatory index
- TG: triglycerides
- TNF-a: tumor necrosis factor alpha
- TSPO: translocator protein
- UPCD: Universitair Psychiatrisch Centrum Duffel (Universitary Psychiatry Hospital of Duffel)
- UZA: Universitair Ziekenhuis Antwerpen (Universitary Hospital of Antwerp)

1. Introduction

1.1. Neuropsychiatric consequences following COVID-19

The coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused more than 183 million cases and 3,96 million deaths to date, since 2020 (1). As the pandemic spread, there has been a growing recognition of neuro-psychiatric (NP) impact of COVID infections. Such complications have been described during the acute phase of the infection with emerging data showing that, particularly in severe cases of COVID-19 infection, the acute viral infection is associated with delirium, confusion, and psychosis, possibly related to the cytokine storm (2). Importantly, a much larger proportion of even mild COVID-19 cases experience neuropsychiatric complications at follow-up. Six months after clinical recovery, an estimated 34% of COVID-19 survivors are diagnosed with new-onset neuropsychiatric disorders, in particular anxiety disorders (17%), mood disorders (14%), substance use disorders (7%) and insomnia (5%) (3). The NICE guidelines have defined acute COVID-19 as signs and symptoms of COVID-19 from the contamination until 4 weeks, ongoing symptomatic COVID-19 from 4 to 12 weeks and post-COVID-19 syndrome for signs and symptoms which persist for more than 12 weeks (4). These long-term post COVID NP symptoms (also referred to as "long COVID", defined as signs and symptoms of COVID-19 after 4 weeks), which are often accompanied by deficits in several cognitive domains (including attention) (5), are unrelated to respiratory insufficiency suggesting independent brain dysfunction. Studies conducted in Italy, Germany, UK and the US found that post COVID NP symptoms seem to affect between 20 to 70% of patients infected with COVID-19 and to last months after respiratory symptoms resolved, suggesting a separate and enduring mechanism (3,6–11). Most strikingly, these NP complications also occur in younger adults with a mild acute COVID-19 presentation (9,10,12).

It is clear that long COVID NP symptoms threaten the quality of life and the societal functioning of a significant proportion of the active population (6,8,13,14). It is estimated that this will severely impact the health of patients infected with COVID-19, long after the pandemic will have ended thanks to vaccination efforts. This may even become a persisting concern if SARS-CoV-2 infection becomes endemic with a pattern analogous to that of flu with Influenza, in particular as the risk of a new neuropsychiatric diagnosis following COVID-19 is 44% greater compared with influenza(3). Despite the major socio-economic consequences of the long-term impact of the pandemic, a standardized way to screen, diagnose, predict and specifically treat patients who develop or will develop these NP symptoms is currently not available. Despite post-viral or post-infectious neuropsychiatric symptomatology has been recognized and studied prior to the COVID-19

pandemic, crucial knowledge about the neurobiological mechanisms underlying these symptoms is still lacking.

1.2. Neurocognitive deficits following COVID-19

Memory and attention problems, reduced information processing and executive dysfunction are among the most frequent complaints of long COVID patients. Specific complaints include concentration difficulties, mental exertion, word finding difficulties, problems with both explicit and procedural memory retrieval (i.e. difficulty completing tasks which used to be "automatic") and problems with planning and organization of daily activities, combined with the subjective experience of "brain fog" (7,15–17).
Scientific evidence is indicating these cognitive deficits are alarmingly widespread among COVID-survivors. In a longitudinal cohort study of 130 Italian patients aged 18-70 years who were assessed with the Brief Assessment of Cognition in Schizophrenia (BACS) (18) at one and three months after acute COVID-19 pneumonia, only 25 patients (19%) showed equivalent scores within the normal range in all cognitive domains (i.e., verbal memory, verbal fluency, working memory, selective attention, processing speed, psychomotor coordination and planning and problem solving), whereas 21 (16%) were poor performers in at least one function, 22 (17%) in two, 18 (14%) in three, 14 (11%) in four, 7 (5%) in five, and 2 (1.5%) demonstrated deficits in all domains (7).

In the study carried out by Miskowiak et al. (17) cognitive functioning was assessed in a group of 29 Danish COVID-patients approximately three to four months after hospitalisation by means of the Screen for Cognitive Impairment in Psychiatry (SCIP) and the Trail Making Test. Fifty-nine % of the patients showed cognitive deficits (38% was globally impaired and 21% was selective impaired), with verbal memory and executive functions being most affected. Ortelli et al. (16) investigated cognitive functions by means of the MOCA, the FAB and three computerized attentive tasks (the vigilance task, the Stroop interference task and the Navon task) in a group of 12 Italian COVID-survivors approximately 9 to 13 weeks from onset of infection. In comparison with a healthy control group, the patient group showed a significant reduced global cognitive functioning (MOCA and FAB) and significant more problems with vigilance and executive attention (computerized attentive tasks).

In the study of Mazza et al. (2121), the presence of psychopathology (mainly depressive symptomatology) was shown to significantly influence neurocognition in post-COVID patients, most notably affecting verbal fluency, information processing, and executive functions at one and/or three months assessments. Patients' sex,

previous psychiatric diagnosis, and duration of hospitalization for COVID-19 did not significantly affect the neurocognitive function (7).

Importantly, preliminary findings of the same research group indicate that the observed cognitive deficits worsen between three- and six-month follow-up (Figure 1, unpublished data). Furthermore, the neurocognitive profile of these patients demonstrates some similarities with patterns observed in depressed patients using the same assessment method. Yet whereas the depressed symptoms of post-COVID NP patients seem to respond well to regular treatment with serotonergic antidepressants, the cognitive symptoms do not.

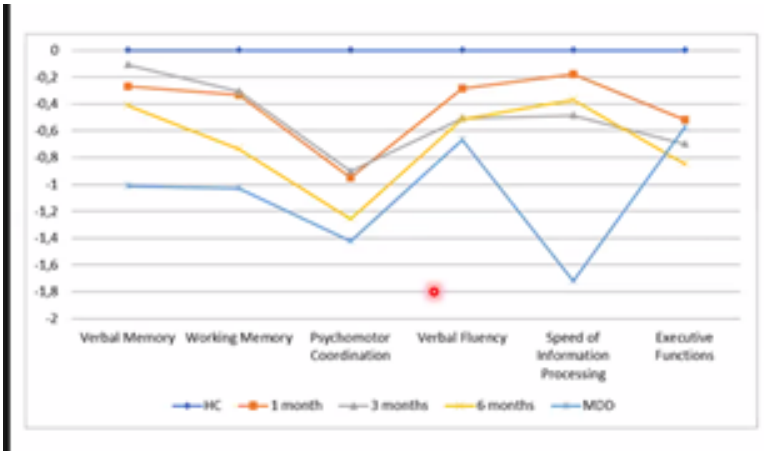

Figure 1: Cognitive profile of post-COVID patients compared to Healthy controls (HC) and depressed patients (MDD) (Mazza et al. 2021, in prep) (7)

1.3. Immune disturbances as translational pathways

1.3.1. Systemic immunological disturbance

Large population cohort studies have replicated the finding of systemic immune disturbances (such as but not limited to IL-6 and C-reactive protein (CRP)) in cohorts of patients with depression (19). Longitudinal studies demonstrated the predictive value of these immunomarkers towards clinical outcome and the development of comorbidities such as chronic fatigue, anorexia, insomnia, somatic disease, … (20–23). Anti-inflammatory agents have been confirmed as valid add-on treatment options to improve depressive symptoms (24). In longitudinal analyses, higher baseline IL-6 levels predicted a subsequent chronic course of depression in women but not in men (25). Similarly, higher baseline IL-6 levels in childhood were associated with depressive and psychotic symptoms at age 18 (26).
Finally, the clinical observation that some patients with long COVID complaints experience an improvement of their NC symptoms following SARS-CoV-2 reinfection or vaccination supports the hypothesis of immune-related pathways involved in the pathophysiology of post-COVID NP complications.

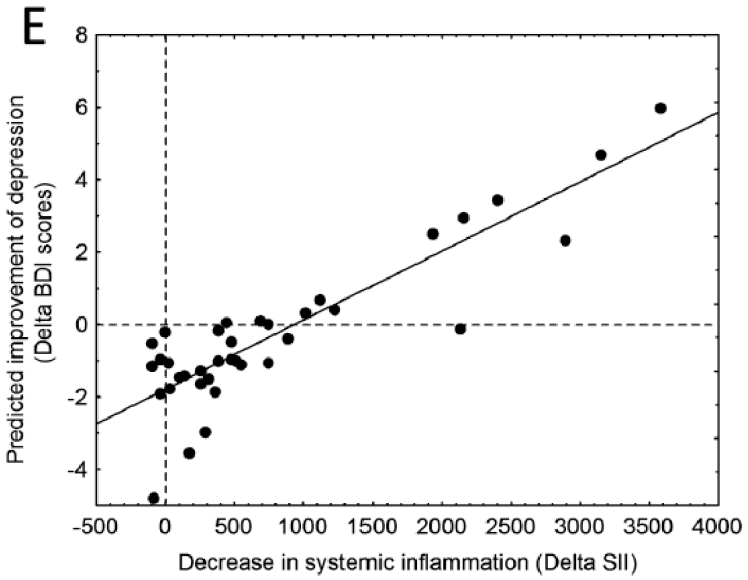

Figure 2: Effect of the decrease of systemic inflammation from hospital admission to 3 months follow-up on the pattern of change of depressive symptoms (measured with BDI-13) during follow-up. (7)

1.3.2. HERV-W-ENV reactivation

It is now clear that COVID-19 NP symptoms can persist beyond 6 months post-infection. Within a window of 90 days, 20% of COVID-survivors are diagnosed with a new psychiatric disorder, which increases to 34% after 6 months (3,11). This suggests that a subsequent autonomous response to the initial infection needs to occur. In this context, a very plausible biological candidate has recently been identified. Indeed, very recent results showed that COVID-19 activates the human endogenous retrovirus-W-envelope protein (HERV-W ENV), which is known to induce persistent innate immune responses and cause neurotoxicity and CNS dysfunction (27). HERV-W elements are atypical segments in the human genome originating from retroviruses, which are able to infiltrate the cell nucleus. Although most HERVs are not transcripted, they encode an envelope protein causing inflammation and neurotoxicity (27). HERV-W-ENV was found to be highly expressed in the lymphocytes of acute COVID-19 patients which was correlated with inflammatory markers (Balestrieri et al, 2021).
Importantly, HERV-W-ENV has previously been implicated in some psychiatric disorders, such as schizophrenia and bipolar disorder (27,28), as well as in neurological conditions such as multiple sclerosis. Importantly, in major mood and psychotic disorders, an inflamed subpopulation represented patients in which the pathology involved HERV-W ENV protein and was associated with increased serum pro-inflammatory cytokines (28).

Another recent publication provided a scientific breakthrough in this domain by identifying the pathogenic pathways induced by HERV-W ENV through altered glutamate functioning and to abnormal behavioural patterns (Johansson et al. 2020). Interestingly, this study evidenced the pivotal role of microglial activation in neurotoxicity and, therefore, highlighted a central common point with the consistent findings of microglial activation as measured with TSPO PET imaging in depression (29).

The reactivation of HERV-W-ENV by SARS-CoV-2 in a proportion of COVID-19 survivors may therefore represent the underlying cause of the persistent systemic inflammation and accompanying neuropsychiatric symptomatology.

1.3.3. Hypothalamo-pituitary-adrenal (HPA) axis function

Long COVID can be regarded as a condition of chronic stress, which could theoretically be associated with disturbances of the hypothalamo-pituitary-adrenal (HPA) axis, although no studies on HPA axis (dys)function in long COVID have been reported yet.
The HPA axis is activated by both physical and psychological stressors and ends with the production of cortisol, which participates in the organism's response to stress, in order to mobilize energy from storages throughout the body and to regulate the psychophysiological, immunological and behavioral responses to stress. The HPA axis activity is regulated by cortisol through negative feedback on the hypothalamus and the pituitary, decreasing the secretion of CRF and ACTH respectively (30–33).
Under normal circumstances, cortisol levels vary across the day, with a peak rise following awakening in the morning (the cortisol awakening response or CAR) and a gradual decline throughout the day. In patients with chronic fatigue syndrome (CFS), an attenuated CAR increase and changes in diurnal cortisol slope (DCS) have been found to be important neuro-endocrine correlates.
Taking the chronic stress and the overlapping symptoms of chronic fatigue and exhaustion into account, one could expect a hypofunction of the HPA axis in long COVID, in analogy with the findings in CFS. Furthermore, there may also be an association with cognitive function and with immunological disturbances (34). Also, the neuro-endocrine correlates might be predictive for the recovery and the response to treatment in long COVID patients. Importantly, HPA axis hyperactivity is well-known to be associated with depression (35).

1.4. Insomnia

Genome Wide Association Studies have demonstrated a strong correlation of the genetic risk of insomnia with depression and anxiety (36,37). Furthermore, insomnia has been identified as a primary risk factor for the onset, persistence and relapse of anxiety, depression and PTSD. In a Network Outcome Analysis of 768 individuals without history of depression, *difficulty initiating sleep* was one of only five direct predictors of first-onset major depressive disorder after six years follow-up (38).
Depending on the study methods, in long COVID patients the prevalence of clinically relevant insomnia was estimated between 5% (diagnosis of insomnia disorder in electronic medical records <6m following infection) and 24% (self-rated insomnia symptoms above clinical cut-off at 3-month follow-up). These symptoms could represent an important risk factor in the subsequent development of anxiety and depressive disorders, and a key mechanism underlying cognitive deficits.

2. Research Objectives

1) Describe the standardized evaluation of the psychopathological and neurocognitive function of long COVID NP patients and their evolution over time;
2) Explore the cross-sectional and longitudinal association between long COVID NP symptoms and immunological measures;
3) Define the prevalence and clinical characteristics of insomnia in long COVID NP patients, as well as its correlation to the clinical psychiatric and neurocognitive symptom domains;
4) Compare the immunological profile of long COVID NP patients to those of existing cohorts of comparable primary psychiatric conditions and healthy controls;
5) To examine the neuro-endocrine (HPA-axis) characteristics of long COVID syndrome

3. Methodology

3.1. Recruitment and study population

Inclusion criteria - patients:
All patients 18-70 years referred to the University Psychiatric Hospital (Campus UZA and Campus Duffel) for psychological and/or cognitive complaints at least >4 weeks following a confirmed diagnosis of COVID-19 infection with a positive PCR test or an antibodies test will be invited to participate in the study. The sample size of the patients is estimated on 50 participants. The patients have to be examined by their

treating physician before enrollment to make sure other medical causes for their complaints are excluded. A positive score on at least two domains (psychological / cognitive) is necessary during the screening phase (see 3.3.1).

Inclusion criteria – controls:
Two control groups, one of 30 healthy controls (Cb), another of 50 COVID-19 survivors without persistent complaints (Ca), matched on age, sex and education with the patient group will be included. The participants for Ca will be recruited in UZA and by advertising. The healthy controls (Cb) will be recruited by mailing within the hospital and by advertising. A positive score on maximum one domain (psychological / cognitive) is allowed in the absence of a psychiatric diagnosis (confirmed by the MINI) and when the complaint causes significant distress (which is investigated during the screening phase).

General exclusion criteria:
Participant is unable to read and understand the consent form and patient-reported outcomes, complete study-related procedures, or communicate with the study staff and informed consent cannot realistically be obtained in retrospect or with the help of a competent family member or legal representative.

Exclusion criteria for each subgroup:

- HPA subgroup
  - Participant is pregnant or breastfeeding
  - Participant receives hormonal replacement therapy (contraception is allowed).
  - Participant is treated with cortisol <4 weeks ago
- Neurocognitive assessment:
  - IQ < 90 (screened with Raven Standard Progressive Matrices (Short Form) (RSPM-SF) (39)
  - Participant takes sedative medication
    - Benzodiazepines: Larger than the equivalent of diazepam 10mg per day. Last administration <8 hours prior to the neurocognitive tests.
    - New sedative antipsychotics/antidepressants (<4 weeks)
    - Other medication from DRUID class III (<4 weeks), last administration <8 hours prior to the neurocognitive tests, or causing significant sedation.

- Severe substance abuse (alcohol + drugs)
- Pre-existing neurological diseases

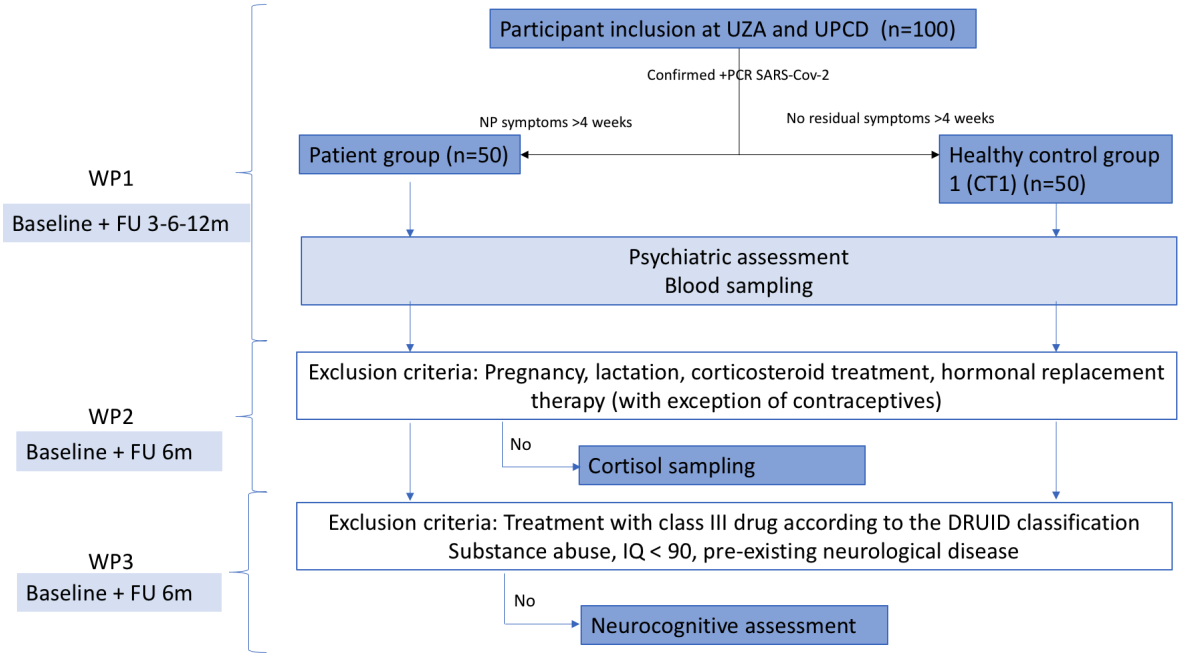

Figure 3: Flowchart of the COVID-19 participant inclusion and the work packages (WP). Participants with a confirmed positive PCR test will be included at UZA or UPCD and are classified in the patient group when suffering from neuropsychiatric symptoms for more than 4 weeks. All participants undergo psychiatric assessment and blood sampling on every time point of the study (WP1). Additionally, if patients do not fulfill the exclusion criteria for HPA-axis assessment (pregnancy, breastfeeding, treatment with corticosteroids, hormonal replacement therapy with exception of contraceptives), they are included in the cortisol subgroup (WP2). Finally, all participants, except those treated with class III drugs according to the DRUID classification, those suffering from pre-existing neurological disease or substance abuse and those with an IQ below 90, undergo neurocognitive assessment (WP3).

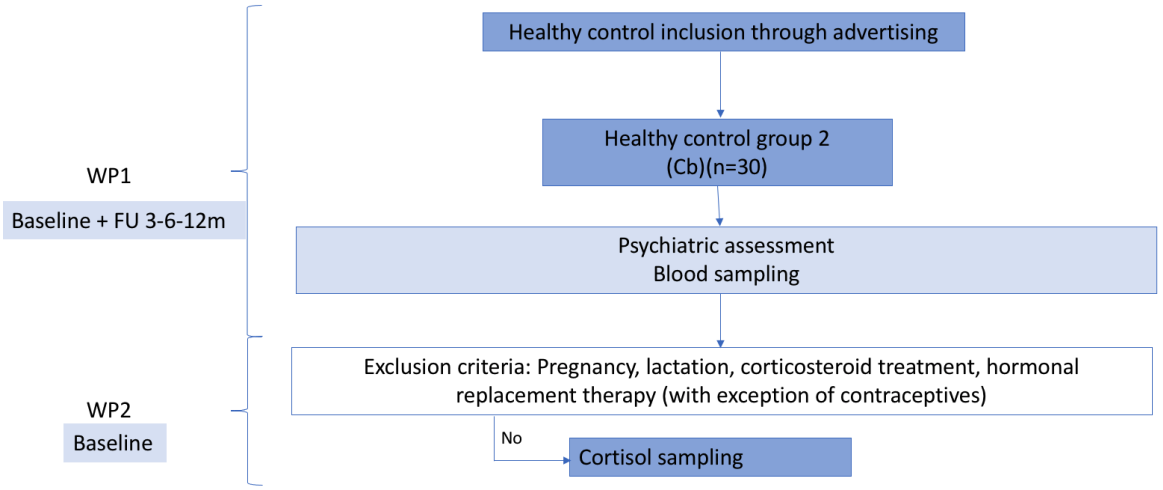

Figure 4: Flowchart of the healthy control inclusion and the work packages (WP). Healthy volunteers can be included in Cb if they were not infected with Sars-CoV-2 in the past and if they do not complain from psychic or cognitive symptoms. If they meet the exclusion criteria for the cortisol testing, they are excluded from the study. WP2 is only performed at baseline.

Study design

General study design:

This is a longitudinal study, which will be organized into three work packages (WP1, WP2 and WP3). Enrollment will continue for an estimated period of 24 months, depending on the time course of the epidemic curve. Last-patient-in is expected no later than November 1st, 2023.

Patients will be referred mainly by the University Hospital of Antwerp (UZA) and enrolled at first visit to the University Psychiatric Hospital (UPCD), where follow-up will also take place at 3, 6 and 12 months. Patients can also be referred by the Flemish long COVID patient support group.

Sample size:

Based on current referral rates, we expect on average 1-2 new referrals each week. After 12 months, we expect a total of ±50 patient inclusions. In a MANOVA analysis, a total sample size of 64 offers 80,0% power to detect a medium sized effect ($f2 \geq 0.15$). We aim for a total sample size of 130 in order to achieve higher effect sizes and to maintain sufficient power in the subgroup analyses and in case of missing values. As Ca is the main control group for the long COVID patient group, these two groups are of equal size (both 50 participants). We included a smaller Cb group to compare the immunological blood parameters between healthy volunteers and long COVID patients.

Study visits:

As part of routine clinical care, patients will receive psychiatric evaluation at the University Hospital Campus UZA, and neurocognitive evaluation at Campus Duffel. During the clinical evaluations, participants will be invited to participate in the study. If they are interested, they will be invited to a baseline visit assessing eligibility and covering informed consent procedure. In order to adhere to social distancing regulations, it may occur that this baseline visit is held digitally via livestream/webcam or by telephone. In this case, patients sign their ICF digitally. To ensure patient privacy, only in-house fully secured internet and telephone connections will be used for distanced baseline visits.

Once included, the participant will be invited for the baseline assessment, which includes a broad neuropsychological examination. A blood sample will be collected at each time point during the study.

	Screening	Baseline	3 months	6 months	12 months
Patient group	Eligibility, psychiatric and IQ screening	Psychiatric assessment Blood sample	Psychiatric assessment Blood sample	Psychiatric assessment Blood sample	Psychiatric assessment Blood sample
+ NC subgroup		Neurocognitive assessment		Neurocognitive assessment	
+ HPA subgroup		Saliva sample		Saliva sample	
Control group A	Eligibility, psychiatric and IQ screening	Psychiatric assessment Blood sample	Psychiatric assessment Blood sample	Psychiatric assessment Blood sample	Psychiatric assessment Blood sample
+ NC subgroup		Neurocognitive assessment			
+ HPA subgroup		Saliva sample			
	Screening	Baseline	1 month	2 months	3 months
Control group B	Eligibility, psychiatric screening and IQ screening	Psychiatric assessment Blood sample Saliva sample	Psychiatric assessment Blood sample	Psychiatric assessment Blood sample	Psychiatric assessment Blood sample

Figure 5: Tasks per (sub)group for each time point of the study

3.3 Study measurements

To begin with, possible participants will be screened for eligibility before inclusion. First, patients with long COVID symptoms can be referred after completing the screening tests, assessing psychological and cognitive complaints, and can be included if these self-report questionnaires indicate neuropsychiatric symptoms. The control group will perform a screening test to exclude any self-reported psychological or cognitive complaints. If possible, these screening tests will be organized on an online platform.

The data which will be collected for this study which are part of routine clinical practice include the following procedures:

- a baseline clinical psychiatric interview to determine a patient's past and current mental state and psychiatric diagnosis, including psychiatric rating scales
- a baseline neuropsychological evaluation including a standardized test battery (optional)
- extensive neurocognitive tests to examine and document persistent cognitive complaints

Additionally, the following study-related procedures will be performed:

- informed consent and eligibility screening
- blood sample draw (max 35ml per visit)
- saliva cortisol sampling
- additional self-rated scales assessing insomnia, fatigue and psychopathology
- a questionnaire regarding important bio-psycho-social factors such as familial psychiatric antecedents, socio-economic status, social network during the pandemic, personal experience with the COVID-related restrictions, …

3.3.1. Psychiatric interview

The psychiatric interview will be performed at baseline by the treating physician as part of standard clinical practice and will include diagnostic evaluation using the MINI. If the MINI was not yet performed by the treating physician, this will be performed during the screening.

Validated self-report questionnaires will be used to assess psychopathology on each time point of the study (see table 1).

Table 1: Neuropsychiatric rating scales

Domain	Scale		Cut-off
SCREENING PHASE (before inclusion)			
Depression, anxiety, trauma	RMT-20	Rapid Measurement Toolkit 20 (40)	MDD ≥ 13 GAD ≥ 11 SAD ≥ 12 Panic ≥ 9 PTSD ≥ 8
Depression	BDI-21	21-item Beck depression inventory (41)	10-18: mild 19-29: moderate 30-63: severe
Anxiety	STAI	State-Trait Anxiety Inventory (42)	State: ≥ 39
Trauma	PCL-5	PTSD Checklist for DSM-5 (44)	≥33
	IES-R	Impact of Event Scale - Revised (45,46)	≥33
Insomnia	ISI	Insomnia Severity Index (47)	≥10
Neurocognitive	CFQ	Cognitive Failure Questionnaire (subjective) (48)	General cut-off score of ≥ 43.
IQ	RSPM-SF	Raven Standard Progressive Matrices (Short Form) (39)	IQ > 90
Substance abuse	ASSIST	Alcohol, Smoking and Substances Involvement Screening Test (72, 73)	≥11 for alcohol, ≥4 for other substances
COVID-19	C19-RehabNeq	COVID-19 Rehabilitation Needs Questionnaire (70)	No cut-off
CLINICAL/EXPERIMENTAL PHASE (baseline – T1 – T2 -T3)			
Diagnosis (only baseline)	MINI	Mini International neuropsychiatric Interview (49)	
Depression	HDRS-17	17-item Hamilton Depression Rating Scale	8-13: mild 14-18: moderate 19-22: severe ≥23: very severe
	BDI-21	21-item Beck depression inventory (41)	
Anxiety	STAI	State-Trait Anxiety Inventory (42)	
Insomnia	PSQI	Pittsburgh Sleep Quality Index (50)	≥5
	ISI	Insomnia Severity Index (47)	

	DRFS	Dream recall frequency scale (exp) (51)	
	ITI	Insomnia Type Inventory (exp)	(only if ISI >9)
Fatigue	FSS	Fatigue Severity Scale (52)	
Trauma	CTQ	Childhood trauma questionnaire (53)	
	IES-R	Impact of Event Scale - Revised (45,46)	≥33
Neurocognitive	DSC	Digit Symbol Coding Subtest from WAIS-IV	see 3.3.2.
	CWI	Color Word Interference Test from the Delis-Kaplan Executive Function System	see 3.3.2.
	BWT	Bourdon-Wiersma Test	
	DS	Digit Span Subtest from WAIS-IV	see 3.3.2.
	RAVLT	Rey Auditory-Verbal Learning Test	see 3.3.2.
	CFT-R	Complex Figure Test (Rey figure)	see 3.3.2.
	BNT-SF	Boston Naming Test, short form	see 3.3.2.
	COWAT	Controlled Oral Word Association Test	see 3.3.2.
	WFT	Word Fluency Test	see 3.3.2.
	S MR	Subtests Similarities from WAIS-IV Subtest Matrix Reasoning from WAIS-IV	see 3.3.2.
	TOL	Tower of London	see 3.3.2.
	TMT	Trail Making Test from the Delis-Kaplan Executive Function System	see 3.3.2.
	PPT	Purdue Pegboard Test	see 3.3.2.
Substance abuse	ASSIST	Alcohol, Smoking and Substances Involvement Screening Test (72, 73)	
COVID-19	PCFS	Post-COVID-19 Functionele Statusschaal (71)	No cutoff

3.3.2. Neuropsychological assessment

COVID-19 patients with persistent complaints of cognitive disabilities (more than 4 weeks post-infection) in daily life are examined with an extensive neuropsychological test battery (see below) at the outpatient clinic of the University Psychiatric Centre in Duffel and at the University Hospital Antwerp. This battery covers a broad range of reliable and valid tests divided into six cognitive domains: attention, memory, visuospatial functions, language, executive functions and psychomotor speed/coordination. The neuropsychological examination lasts an average of 2 hours per participant. The tests are carried out by the

same experienced neuropsychologist and also follow a standardized sequence. In addition to the neuropsychological assessment, two self-report scales are filled out by each patient. These scales are used to detect possible complaints of fatigue and possible cognitive problems which are experienced by the patient during daily life activities.

A control group of on average 50 COVID-19 survivors without persistent complaints (Ca), matched on age and years of education with the patient group, will also undergo the neuropsychological test battery.

- *Attention*
- The Bourdon-Wiersma Test (BWT) (54) was used to evaluate sustained attention. The primary outcome variables are mean rule time and total omissions.
- The Digit Symbol Coding Subtest from the Wechsler Adult Intelligence Scale (4th Edition) (WAIS-IV (DSC)) (58) is used to assess divided attention and speed of information processing. The total correct symbols and the total errors (or incorrect symbols) within the allowed time of 120 s are used as outcome measures.
- The Dutch version of the Color Word Interference Test from the Delis-Kaplan Executive Function System (D-KEFS (CWI)) (65) is administered to measure focused attention and inhibition of interference (card III) and alternating attention and cognitive flexibility (card IV). The total raw score (total time) and the total uncorrected errors for card III (evaluation of focused attention) and card IV (evaluation of alternating attention) are used as outcome measures
- *Memory*

  The Digit Span Subtest from the Wechsler Adult Intelligence Scale (4th Edition) (WAIS-IV (DS)) (58) is used to evaluate working memory. For the digit span forward and the digit span backward we use the total raw score and the longest span (forward or backward) as outcome measures.

  The Rey Auditory-Verbal Learning Test (RAVLT) (54,55) is administered to evaluate auditory-verbal memory for unrelated information. The variables we use for the analysis are total immediate recall (over 5 trials) (the maximum learning score is 75), long-term delayed recall (after a 20-min time interval; the

maximum recall score is 15) and long-term delayed recognition (after a 20-min time interval; the maximum recognition score is 15).

Visuospatial memory for related information is assessed by means of the Complex Figure Test (Rey figure) (CFT-R) (54–56). The two measures we use are immediate recall and long-term delayed recall (after a 20-min time interval) of recently learned information.

- *Visuospatial functions*

  The copy of the Complex Figure Test (Rey figure) (RCFT-R) (54–56) is administered to evaluate visuospatial judgement. The total score (with a maximum of 36) is recorded.

- *Language*

  In our study we administer the short form of the Boston Naming Test (BNT-SF) (66) to measure word finding (i.e., naming of 29 objects). The outcome measure is the total correct answers (with a maximum of 29).

- *Executive functions*

  The Controlled Oral Word Association Test (57) and the Word Fluency Test (WFT) (67) are used to assess phonetic verbal fluency and semantic verbal fluency, respectively. The total words generated is used as outcome measure for both tests.

  The Subtests Similarities and Matrix Reasoning from the Wechsler Adult Intelligence Scale (4th Edition) (WAIS-IV (S) and WAIS-IV (MR)) (58) are selected to measure verbal abstract reasoning and non-verbal abstract reasoning, respectively. The total raw score is used as outcome measure for both subtests.

  The Tower of London Test (59) is used to evaluate planning and problem solving. The outcome variables are the total correct score, the total move score and the total problem-solving time.

- *Psychomotor speed/coordination*

  The conditions 4 and 5 of the Trail Making Test from the Delis-Kaplan Executive Function System (D-KEFS (TMT)) (68) are administered to evaluate psychomotor speed with a cognitive component (cognitive flexibility) (condition 4) and psychomotor speed without a cognitive component (condition 5). For both conditions, the total raw score (total time) is used as outcome measure.

  The Purdue Pegboard Test (60) is used to assess psychomotor coordination. The total raw score (or the average of three trials, each trial with a time limit of 30 s) for the preferred hand, for the

non-preferred hand and for both hands are used as outcome measures.

- *Cognitive functioning in daily life*
  The Cognitive Failure Questionnaire (CFQ) (69) is used to detect possible cognitive problems (or failures) which are noticed by patients during daily life activities in the past four weeks. This self-report scale consists of 25 items with a rating of 0 (never), 1 (rarely), 2 (sometimes), 3 (often) or 4 (very often) for each item. The total score (with a maximum of 100) is used as outcome measure.
- *Fatigue*
  The Fatigue Severity Scale (FSS) (61) is used to detect possible complaints of fatigue in daily life by the patient. This self-report scale consists of 9 items about fatigue, its severity and how it can affect several daily activities. The items are scored on a 7-point rating scale from 1 (strongly disagree) to 7 (strongly agree). The total score is used as outcome measure (with a maximum of 63).
- Cognitive rehabilitation
  The COVID-19 patients are divided into two groups: a treatment group and a non-treatment group. The patients in the treatment group follow a cognitive rehabilitation program. This program consists of 12 group sessions of one hour (one session per week). A maximum of 6 patients participate to a group session. Each session consists of two cognitive rehabilitation methods. These are restorative therapy (which means administering several cognitive exercises (with increased level of complexity) in order to improve the specific cognitive deficits of each individual patient (as was detected by means of the neuropsychological baseline evaluation) with regard to attention, memory and/or executive functions) and (2) compensatory therapy (which means teaching compensation strategies in order to cope better with the cognitive disabilities concerning attention, memory and/or executive functions that each individual patient experiences during daily life activities). The patients in the non-treatment group receive no cognitive rehabilitation program.
- Neuropsychological reassessment
  The patients in both groups (treatment group versus non-treatment group) undergo a neuropsychological reassessment after approximately 3 months. For some tests (RAVLT, CFT-T (Taylor figure), COWAT and WFT) a parallel

version is used to minimize a possible practice effect. Of course, for the treatment group this neuropsychological reassessment is necessary to evaluate the progression of cognitive functioning after 3 months of cognitive rehabilitation.

3.3.3. Blood sampling

Blood samples will be collected in a standardized manner by venipuncture. Two blood vials will be collected and processed to perform the following analyses:

- serum collection tube(s) allowing for HERV-W ENV and serological assays
- plasma collection tubes for the assessment of other immune-related biomarkers (including but not limited to CRP, IL-6, IL-1b, TNF-a, IFN-g, tryptophan, kynurenine, 3-OH-kynurenine, quinolinic acid, kynurenic acid, enzymes of the kynurenine pathway).

A maximum of 35ml blood will be collected during each visit, clinical test sampling included. Study-dedicated blood tubes will be processed for respective plasma and serum extraction according to standardized protocols and plasma / serum will subsequently be stored at -80°C until analysis or elimination.

Residual pellet material of whole blood centrifugation for plasma collection will be utilized for DNA extraction to perform additional genotyping. Emerging evidence has linked specific DNA haplotypes with increased risk of psychiatric morbidity. Exploration of specific immune related genes in post-COVID NP patients might reveal insights in genetic predisposition for infection-driven psychiatric decompensation. DNA sequencing of immune related loci (including but not limited to MHC-II) will be performed on residual pellet material from centrifugated blood collection tubes for plasma procurement if specific additional consent is obtained from the participant. If such consent is not granted, residual pellet material will be discarded after plasma collection.

3.3.4. Cortisol measurements (HPA axis)

Salivary cortisol measures will be used as functional measure of the HPA axis activity, as the unbound cortisol fraction in saliva is highly correlated to plasma cortisol (62–64). Baseline diurnal cortisol and CAR levels will be measured using saliva, collected at home with Salivette® (Sarstedt) synthetic swabs. The rationale behind the choice of home sampling, is to maximize normal circumstances and to minimize stress related to the measuring procedure. Participants are also asked to perform the sampling on two consecutive days with no anticipated excessive stress or physical or mental efforts. The Salivette® sampling method is easy and minimally invasive. Participants put the swabs in their mouth and move them around for

approximately 60 seconds. Participants are required to refrain from smoking, drinking and eating or brushing the teeth 30 minutes before each measurement. The sampling times are immediately upon awakening, 30, 45 and 60 minutes after awakening (CAR). All samples will be analyzed through electrochemiluminescence immunoassay at the laboratory of the Antwerp University Hospital. The lower detection limit of cortisol is 0.544 ng/ml. This value is assigned whenever cortisol levels were below the detection threshold.

3.3.5. Electronic clinical records

The following demographic and clinical variables will be collected through electronic clinical records. If certain data is unavailable from the records, the participant may be contacted by a member of the research team for an additional interview.

- Demographics: age, gender
- Clinical diagnosis at baseline psychiatric evaluation
- Medical history
- Psychiatric history
- Family psychiatric history
- Current + past medication use
- Metabolic parameters: weight, length, waist circumference, triglycerides (TG), HDL-cholesterol, blood pressure, fasting plasma glucose
- Current substance abuse: nicotine, alcohol, cannabis
- If available, results of RT-PCR of SARS-Cov-2 on a nasopharyngeal swab or of SARS-Cov-2 serology
- Date of acute infection, duration of NP symptoms of long Covid
- If available, brain imaging, polysomnography or other technical exams performed during or after COVID-19 infection, including lab results during acute COVID-19 infection
- If available: inflammatory parameters in lab result during acute infection. Otherwise: inflammatory parameters of last clinical lab result (including but not limited to white blood cell count)
- SARS-Cov-2 vaccination status

3.3.6. Follow-up measures

Depending on the clinical findings, treatment with psychopharmacological compounds may be initiated or patients may be enrolled in a group cognitive remediation program contingent upon clinical indication. Follow-up visits with psychiatric evaluation, self-rating scales and blood sampling will take place every 3

months for a maximum of one year. Follow-up neuropsychological assessment will take place once after 6 months.

3.4. Endpoint measurements

"NP symptoms"
- Baseline and follow-up scores on clinical scales of depression, anxiety, and insomnia
- (subgroup) Baseline and follow-up cognitive functioning

"Inflammation"
- Quantitative measurement of HERV-W-ENV in serum at baseline
- Calculation of systemic immune inflammatory index (SII= thrombocyte count x neutrophile count / lymphocyte count) at baseline and follow-up
- Quantitative analysis of CRP and IL-6 at baseline and follow-up
- Quantitative analysis of kynurenine metabolites at baseline and follow-up

(subgroup) "HPA axis"
- CAR

3.5. Statistical analysis

CAPRI has ongoing collaborations with StatUA and BIOMINA for statistical analyses of past and ongoing projects involving biomarker-data. In this project, we will study cross-sectional and bidirectional longitudinal associations between *NP symptoms* and *Inflammation* or *Insomnia* (cfr above).
To study the cross-sectional association using all available data, we will stack data from all timepoints in one cross-sectional dataset. To account for the fact that one person can have multiple observations in this combined cross-sectional dataset, we will analyze the association between NP symptoms and inflammation / insomnia (measured at the same timepoint) using linear mixed models with timepoint as repeated effect and patient identifier as within-subject effect and using an unstructured correlation matrix. Estimates from this model thus represent the cross-sectional association between predictor and outcome.
For longitudinal analyses the effect of baseline *Inflammation* and *Insomnia* on *NP symptoms* at 3- and 6-month follow-up will be evaluated using linear mixed models with time as repeated effect and patient identifier as within-subject effect and using an unstructured correlation matrix. Baseline predictor by time interaction (time coded as 0, 3, or 6) will be included to test differences in slope but retained only in models when statistically significant. Treatment group (antidepressant treatment, insomnia treatment, cognitive remediation, a combination, or none) will be included

as predictor. Baseline values of NP symptoms will be included as covariates, and all other covariates measured at baseline as well.

3.6. Management of the database

Management of the database will be performed in line with applicable regulatory procedures by SINAPS (research unit of CAPRI and Emmaus in UPC Duffel). The data manager of the study is Dr. Katrien Skorobogatov. The database, only including pseudonymized (encoded) data, will be stocked on an encrypted hard drive, accessible by the SINAPS team members. The file containing information on the coding of the participants will be kept in a separate file, equally kept on an encrypted hard drive, only accessible with the encryption key by the principal investigator (Dr. Livia De Picker). The research data will be archived for a duration of 20 years after the study is completed.

3.7. Ethical aspects

The study is submitted to the ethics committee of Antwerp University Hospital / University of Antwerp and will be conducted in accordance with the declaration of Helsinki. Written informed consent is necessary before inclusion and participation.
All participants will be informed about the no-fault insurance which covers any personal damage directly or indirectly related to the study.

The participants will receive a fee of €50 for each visit to compensate for any financial costs they may have suffered related to their participation in this study (e.g., for transportation to and from the hospital), except for patients who participate in the neurocognitive tests. Instead, patients undergoing the neurocognitive tests will receive these assessments including a full report for free.

In order to clearly separate the research project from clinical treatment, which takes place in UZA, the participants will be invited to UPCD for every time point in the study.
All participants have the right to withdraw from the study at any moment for any reason, since participation is strictly voluntary. The researcher has the right to withdraw a participant for any of the following reasons:

- Withdrawal of consent
- Coöperation inability
- Protocol violation

References

1. COVID Live Update: 185,088,334 Cases and 4,003,549 Deaths from the Coronavirus - Worldometer [Internet]. [cited 2021 Jul 6]. Available from: https://www.worldometers.info/coronavirus/

2. Rogers JP, Chesney E, Oliver D, Pollak TA, McGuire P, Fusar-Poli P, et al. Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review and meta-analysis with comparison to the COVID-19 pandemic. Lancet Psychiatry. 2020 Jul;7(7):611–27.

3. Taquet M, Luciano S, Geddes JR, Harrison PJ. Bidirectional associations between COVID-19 and psychiatric disorder: retrospective cohort studies of 62 354 COVID-19 cases in the USA. Lancet Psychiatry. 2021 Feb;8(2):130–40.

4. COVID-19 rapid guideline: managing the long-term effects of COVID-19 (NG188): Evidence reviews 6 and 7: monitoring and referral. London: National Institute for Health and Care Excellence (UK); 2021.

5. Nakamura ZM, Nash RP, Laughon SL, Rosenstein DL. Neuropsychiatric Complications of COVID-19. Curr Psychiatry Rep. 2021 Mar 16;23(5):25.

6. De Lorenzo R, Conte C, Lanzani C, Benedetti F, Roveri L, Mazza MG, et al. Residual clinical damage after COVID-19: A retrospective and prospective observational cohort study. PLoS One. 2020 Oct 14;15(10):e0239570.

7. Persistent psychopathology and neurocognitive impairment in COVID-19 survivors: Effect of inflammatory biomarkers at three-month follow-up. Brain Behav Immun. 2021 May 1;94:138–47.

8. Varatharaj A, Thomas N, Ellul MA, Davies NWS, Pollak TA, Tenorio EL, et al. Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study. Lancet Psychiatry. 2020 Oct;7(10):875–82.

9. Augustin M, Schommers P, Stecher M, Dewald F, Gieselmann L, Gruell H, et al. Post-COVID syndrome in non-hospitalised patients with COVID-19: a longitudinal prospective cohort study. Lancet Reg Health Eur. 2021 Jul;6:100122.

10. Woo MS, Malsy J, Pöttgen J, Seddiq Zai S, Ufer F, Hadjilaou A, et al. Frequent neurocognitive deficits after recovery from mild COVID-19. Brain Commun. 2020 Nov 23;2(2):fcaa205.

11. Taquet M, Geddes JR, Husain M, Luciano S, Harrison PJ. 6-month neurological and psychiatric outcomes in 236 379 survivors of COVID-19: a retrospective cohort study using electronic health records. Lancet Psychiatry. 2021 May;8(5):416–27.

12. Hampshire A, Trender W, Chamberlain SR, Jolly A, Grant JE, Patrick F, et al. Cognitive deficits in people who have recovered from COVID-19 relative to controls: An N=84,285 online study [Internet]. Available from: http://dx.doi.org/10.1101/2020.10.20.20215863

13. Ma Y-F, Li W, Deng H-B, Wang L, Wang Y, Wang P-H, et al. Prevalence of depression and its association with quality of life in clinically stable patients with COVID-19. J Affect Disord. 2020 Oct 1;275:145–8.

14. Mazza MG, De Lorenzo R, Conte C, Poletti S, Vai B, Bollettini I, et al. Anxiety and depression in COVID-19 survivors: Role of inflammatory and clinical predictors [Internet]. Vol. 89, Brain, Behavior, and Immunity. 2020. p. 594–600. Available from: http://dx.doi.org/10.1016/j.bbi.2020.07.037

15. Almeria M, Cejudo JC, Sotoca J, Deus J, Krupinski J. Cognitive profile following COVID-19 infection: Clinical predictors leading to neuropsychological impairment. Brain Behav Immun Health. 2020 Dec;9:100163.

16. Ortelli P, Ferrazzoli D, Sebastianelli L, Engl M, Romanello R, Nardone R, et al. Neuropsychological and neurophysiological correlates of fatigue in post-acute patients with neurological manifestations of COVID-19: Insights into a challenging symptom. J Neurol Sci. 2021 Jan 15;420:117271.

17. Miskowiak KW, Johnsen S, Sattler SM, Nielsen S, Kunalan K, Rungby J, et al. Cognitive impairments four months after COVID-19 hospital discharge: Pattern, severity and association with illness variables. Eur Neuropsychopharmacol. 2021 May;46:39–48.

18. Keefe RSE, Goldberg TE, Harvey PD, Gold JM, Poe MP, Coughenour L. The Brief Assessment of Cognition in Schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. Schizophr Res. 2004 Jun 1;68(2-3):283–97.

19. CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies. J Affect Disord. 2013 Sep 25;150(3):736–44.

20. Maes M, Bosmans E, Suy E, Vandervorst C, De Jonckheere C, Raus J. Immune Disturbances during Major Depression: Upregulated Expression of Interleukin-2 Receptors [Internet]. Vol. 24, Neuropsychobiology. 1990. p. 115–20. Available from: http://dx.doi.org/10.1159/000119472

21. Dantzer R. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. Eur J Pharmacol. 2004 Oct 1;500(1-3):399–411.

22. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008 Jan;9(1):46–56.

23. Maes M, Kubera M, Obuchowiczwa E, Goehler L, Brzeszcz J. Depression's multiple comorbidities explained by (neuro)inflammatory and oxidative &

nitrosative stress pathways. Neuro Endocrinol Lett. 2011;32(1):7–24.

24. Nettis MA, Lombardo G, Hastings C, Zajkowska Z, Mariani N, Nikkheslat N, et al. Augmentation therapy with minocycline in treatment-resistant depression patients with low-grade peripheral inflammation: results from a double-blind randomised clinical trial. Neuropsychopharmacology. 2021 Apr;46(5):939–48.

25. Lamers F, Milaneschi Y, Smit JH, Schoevers RA, Wittenberg G, Penninx BWJH. Longitudinal Association Between Depression and Inflammatory Markers: Results From the Netherlands Study of Depression and Anxiety. Biol Psychiatry. 2019 May 15;85(10):829–37.

26. Khandaker GM, Stochl J, Zammit S, Goodyer I, Lewis G, Jones PB. Childhood inflammatory markers and intelligence as predictors of subsequent persistent depressive symptoms: a longitudinal cohort study. Psychol Med. 2018 Jul;48(9):1514–22.

27. Perron H, Hamdani N, Faucard R, Lajnef M, Jamain S, Daban-Huard C, et al. Molecular characteristics of Human Endogenous Retrovirus type-W in schizophrenia and bipolar disorder. Transl Psychiatry. 2012 Dec 4;2:e201.

28. Tamouza R, Meyer U, Foiselle M, Richard J-R, Lu C-L, Boukouaci W, et al. Identification of inflammatory subgroups of schizophrenia and bipolar disorder patients with HERV-W ENV antigenemia by unsupervised cluster analysis [Internet]. Vol. 11, Translational Psychiatry. 2021. Available from: http://dx.doi.org/10.1038/s41398-021-01499-0

29. Markers of central inflammation in major depressive disorder: A systematic review and meta-analysis of studies examining cerebrospinal fluid, positron emission tomography and post-mortem brain tissue. Brain Behav Immun. 2019 Oct 1;81:24–40.

30. Chrousos GP. Stress and disorders of the stress system. Nat Rev Endocrinol. 2009 Jul;5(7):374–81.

31. Cacioppo JT, Burleson MH, Poehlmann KM, Malarkey WB, Kiecolt-Glaser JK, Berntson GG, et al. Autonomic and neuroendocrine responses to mild psychological stressors: effects of chronic stress on older women. Ann Behav Med. 2000 Spring;22(2):140–8.

32. Cassiers LLM, Niemegeers P, Fransen E, Morrens M, De Boer P, Van Nueten L, et al. Neuroendocrine and Inflammatory Effects of Childhood Trauma Following Psychosocial and Inflammatory Stress in Women with Remitted Major Depressive Disorder. Brain Sciences. 2019 Dec 13;9(12):375.

33. Miller GE, Chen E, Zhou ES. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans [Internet]. Vol. 133, Psychological Bulletin. 2007. p. 25–45. Available from: http://dx.doi.org/10.1037/0033-2909.133.1.25

34. Irwin MR, Slavich GM, Cacioppo JT, Tassinary LG, Berntson GG. Psychoneuroimmunology. In: Handbook of Psychophysiology. Cambridge University Press; 2016. p. 377–98.

35. Stetler C, Miller GE. Depression and Hypothalamic-Pituitary-Adrenal Activation: A Quantitative Summary of Four Decades of Research [Internet]. Vol. 73, Psychosomatic Medicine. 2011. p. 114–26. Available from: http://dx.doi.org/10.1097/psy.0b013e31820ad12b

36. Wing YK, Zhang J, Lam SP, Li SX, Tang NL, Lai KY, et al. Familial aggregation and heritability of insomnia in a community-based study. Sleep Med. 2012 Sep;13(8):985–90.

37. Lind MJ, Hawn SE, Sheerin CM, Aggen SH, Kirkpatrick RM, Kendler KS, et al. An examination of the etiologic overlap between the genetic and environmental influences on insomnia and common psychopathology. Depress Anxiety. 2017 May;34(5):453–62.

38. Blanken TF, Borsboom D, Penninx BW, Van Someren EJ. Network outcome analysis identifies difficulty initiating sleep as a primary target for prevention of depression: a 6-year prospective study. Sleep [Internet]. 2020 May 12;43(5). Available from: http://dx.doi.org/10.1093/sleep/zsz288

39. Raven JC. Raven Standard Progressive Matrices [Internet]. PsycTESTS Dataset. 2016. Available from: http://dx.doi.org/10.1037/t07027-000

40. Batterham PJ, Sunderland M, Carragher N, Calear AL. Development of the RMT20, a composite screener to identify common mental disorders. BJPsych Open. 2020 May 18;6(3):e50.

41. Beck AT. An Inventory for Measuring Depression [Internet]. Vol. 4, Archives of General Psychiatry. 1961. p. 561. Available from: http://dx.doi.org/10.1001/archpsyc.1961.01710120031004

42. Vigneau F, Cormier S. The factor structure of the State-Trait Anxiety Inventory: an alternative view. J Pers Assess. 2008 May;90(3):280–5.

43. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: Psychometric properties [Internet]. Vol. 56, Journal of Consulting and Clinical Psychology. 1988. p. 893–7. Available from: http://dx.doi.org/10.1037/0022-006x.56.6.893

44. Boeschoten MA, Van der Aa N, Bakker A, Ter Heide FJJ, Hoofwijk MC, Jongedijk RA, et al. Dutch Clinician-Administered PTSD Scale for DSM-5 [Internet]. PsycTESTS Dataset. 2019. Available from: http://dx.doi.org/10.1037/t70563-000

45. Mouthaan J, Sijbrandij M, Reitsma JB, Gersons BPR, Olff M. Comparing Screening Instruments to Predict Posttraumatic Stress Disorder [Internet]. Vol. 9, PLoS ONE. 2014. p. e97183. Available from:

http://dx.doi.org/10.1371/journal.pone.0097183

46. Weiss DS. The Impact of Event Scale: Revised [Internet]. Cross-Cultural Assessment of Psychological Trauma and PTSD. p. 219–38. Available from: http://dx.doi.org/10.1007/978-0-387-70990-1_10

47. Morin CM, Belleville G, Bélanger L, Ivers H. The Insomnia Severity Index: psychometric indicators to detect insomnia cases and evaluate treatment response. Sleep. 2011 May 1;34(5):601–8.

48. Broadbent DE, Cooper PF, FitzGerald P, Parkes KR. The Cognitive Failures Questionnaire (CFQ) and its correlates. Br J Clin Psychol. 1982 Feb;21(1):1–16.

49. Lecrubier Y, Sheehan D, Hergueta T, Weiller E. The mini international neuropsychiatric interview [Internet]. Vol. 13, European Psychiatry. 1998. p. 198S – 198S. Available from: http://dx.doi.org/10.1016/s0924-9338(99)80239-9

50. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research [Internet]. Vol. 28, Psychiatry Research. 1989. p. 193–213. Available from: http://dx.doi.org/10.1016/0165-1781(89)90047-4

51. Schredl M. Reliability and stability of a dream recall frequency scale. Percept Mot Skills. 2004 Jun;98(3 Pt 2):1422–6.

52. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol. 1989 Oct;46(10):1121–3.

53. Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T, et al. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. Child Abuse Negl. 2003 Feb;27(2):169–90.

54. Bouma A. Handboek neuropsychologische diagnostiek. 2012. 863 p.

55. Lezak MD, Howieson DB, Bigler ED, Tranel D. Neuropsychological Assessment. OUP USA; 2012. 1161 p.

56. Meyers JE, Meyers KR. Rey complex figure test and recognition trial: manual. 1999. 73 p.

57. Patterson J. Controlled Oral Word Association Test [Internet]. Encyclopedia of Clinical Neuropsychology. 2011. p. 703–6. Available from: http://dx.doi.org/10.1007/978-0-387-79948-3_876

58. Wechsler D. Wechsler Adult Intelligence Scale--Fourth Edition [Internet]. PsycTESTS Dataset. 2012. Available from: http://dx.doi.org/10.1037/t15169-000

59. Culbertson WC, Zillmer EA. Tower of LondonDX™ 2nd Edition [Internet].

PsycTESTS Dataset. 2012. Available from: http://dx.doi.org/10.1037/t05076-000

60. Strauss E, Sherman EMS, Spreen O. A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary. American Chemical Society; 2006. 1216 p.

61. Krupp LB, Pollina DA. Mechanisms and management of fatigue in progressive neurological disorders [Internet]. Vol. 9, Current Opinion in Neurology. 1996. p. 456–60. Available from: http://dx.doi.org/10.1097/00019052-199612000-00011

62. Lovallo WR, Buchanan TW, Cacioppo JT, Tassinary LG, Berntson GG. Stress Hormones in Psychophysiological Research: Emotional, Behavioral, and Cognitive Implications. In: Handbook of Psychophysiology. Cambridge University Press; 2016. p. 465–94.

63. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology. 1994;19(4):313–33.

64. Wedekind D, Bandelow B, Broocks A, Hajak G, Rüther E. Salivary, total plasma and plasma free cortisol in panic disorder. J Neural Transm. 2000;107(7):831–7.

65. Delis D, Kaplan E, Kramer J (2008) Delis-Kaplan Executive Function System: Color-Word Interference Test - Nederlandstalige bewerking. Pearson, Amsterdam.

66. Roomer E, Brok S, Hoogerwerf A, Linn D (2011) Handleiding Boston Benoem Taak - een test voor woordvinding. Hogeschool Utrecht, Utrecht.

67. Mulder J, Dekker P, Dekker R (2014) Woord-fluencytest en figuur-fluencytest. Hogrefe Uitgevers, Amsterdam.

68. Delis D, Kaplan E, Kramer J (2008) Delis-Kaplan Executive Function System: Trail Making Test - Nederlandstalige bewerking. Pearson, Amsterdam.

69. Ponds R, van Boxtel M, Jolles J (2006) De 'Cognitive Failure Questionnaire' als maat voor subjectief cognitief functioneren. Tijdschrift voor neuropsychologie, 1(2): 37-42.

70. Lemhöfer et al. Zufriedenheit COVID-19-Erkrankter mit den Akteuren des Gesundheitssystems un der rehabilitativen Threapieversorgung unter Verwendung des COVID-19-Rehabilitation Needs Questionnaire (RehabNeQ) in Bayern. Phys Med Rehab Kuror 2021 DOI: 10.1055/a-1528-1667

71. Machado, F.V.C., Meys, R., Delbressine, J.M. *et al.* Construct validity of the Post-COVID-19 Functional Status Scale in adult subjects with COVID-19. *Health Qual Life Outcomes* 19, 40 (2021). https://doi.org/10.1186/s12955-021-01691-2

72. Humeniuk, R., Ali, R., Babor, T. F., Farrell, M., Formigoni, M. L., (....), & Simon, R. (2008). Validation of the alcohol, smoking and substance involvement screening test (ASSIST). Addiction. doi: 10.1111/j.1360-0443.2007/02114.x.

73. VAD (2010). Handleiding ASSIST voor de eerstelijnsgezondheidszorg. Brussel: VAD.