BMJ Open

Neurobiological basis and risk factors of persistent fatigue and concentration problems after COVID-19: study protocol for a prospective case–control study (VeCosCO)

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To cite: Verveen A, Verfaillie SCJ, Visser D, *et al*. Neurobiological basis and risk factors of persistent fatigue and concentration problems after COVID-19: study protocol for a prospective case–control study (VeCosCO). *BMJ Open* 2023;**13**:e072611. doi:10.1136/bmjopen-2023-072611

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2023-072611).

Received 07 February 2023
Accepted 08 June 2023

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ABSTRACT

Introduction The risk factors for persistent fatigue and cognitive complaints after infection with SARS-CoV-2 and the underlying pathophysiology are largely unknown. Both clinical factors and cognitive-behavioural factors have been suggested to play a role in the perpetuation of complaints. A neurobiological aetiology, such as neuroinflammation, could be the underlying pathophysiological mechanism for persisting complaints. To unravel factors associated with persisting complaints, VeCosCO will compare individuals with and without persistent fatigue and cognitive complaints >3 months after infection with SARS-CoV-2. The study consists of two work packages. The first work package aims to (1) investigate the relation between persisting complaints and neuropsychological functioning; (2) determine risk factors and at-risk phenotypes for the development of persistent fatigue and cognitive complaints, including the presence of postexertional malaise and (3) describe consequences of persistent complaints on quality of life, healthcare consumption and physical functioning. The second work package aims to (1) determine the presence of neuroinflammation with [^{18}F]DPA-714 whole-body positron emission tomography (PET) scans in patients with persisting complaints and (2) explore the relationship between (neuro)inflammation and brain structure and functioning measured with MRI.

Methods and analysis This is a prospective case–control study in participants with and without persistent fatigue and cognitive complaints, >3 months after laboratory-confirmed SARS-CoV-2 infection. Participants will be mainly included from existing COVID-19 cohorts in the Netherlands covering the full spectrum of COVID-19 acute disease severity. Primary outcomes are neuropsychological functioning, postexertional malaise, neuroinflammation measured using [^{18}F]DPA-714 PET, and brain functioning and structure using (f)MRI.

Ethics and dissemination Work package 1 (NL79575.018.21) and 2 (NL77033.029.21) were approved by the medical ethical review board of the Amsterdam University Medical Centers (The Netherlands). Informed consent is required prior to participation in the study. Results of this study will be submitted for publication in peer-reviewed journals and shared with the key population.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Recruitment via existing prospective cohorts thereby limiting self-selection bias and ensuring the availability of prospectively collected data prior to enrollment in this study.
⇒ Inclusion of participants covering the full spectrum of COVID-19 disease severity in the acute phase of infection.
⇒ Collecting and studying an extensive set of biopsychosocial factors and extensive imaging data that are hypothesised to be related with persistent complaints after COVID-19.
⇒ The inability to determine with certainty whether self-reported symptoms are a direct consequence of COVID-19, were present prior to COVID-19 or developed after COVID-19 due to other causes (ie, background prevalence).
⇒ The inability to discriminate between different SARS-CoV-2 variants.

INTRODUCTION

Persistent fatigue is one of the most prevalent complaints after infection with SARS-CoV-2, with prevalence rates of 15%–45% up to 12 months after infection,[1–4] together with cognitive complaints[5–9] and postexertional malaise (PEM).[10 11] Persisting complaints have been coined as 'long COVID' or postacute sequelae of COVID-19 (PASC). The underlying pathophysiology and risk factors for the development of PASC are largely unknown. Symptoms resemble those of other postinfection syndromes such as Q-fever fatigue syndrome and Post-Lyme disease syndrome.[12] Based on the literature about other infections, clinical and laboratory markers (reflecting disease severity), as well as cognitive and behavioural responses to symptoms may be risk factors for the development of persistent fatigue and cognitive complaints following infection with SARS-CoV-2.[2 13]

It has been demonstrated that a substantial number of COVID-19 patients show cognitive impairments on global cognitive tests 12–26 weeks after diagnosis.[14 15] Most studies, however, report on self-reported cognitive complaints or global cognitive function (non-normative), rather than specific objective cognitive performance measured with neuropsychological assessment. A large population-based study found cognitive deficits in long COVID with respect to reasoning, problem solving, spatial planning and target detection, but spared working-memory span, emotional processing and simpler functions in the early-chronic phase (<1 year).[16] Neuropsychological studies showed pronounced memory impairments 6 months,[17] and impaired attention/processing, language, executive and visuospatial functioning 1 year after infection.[18] Notwithstanding, while many individuals report long-term subjective cognitive deficits, long-term (>2 years) objective cognitive functioning after COVID-19 has not thoroughly been investigated yet.

Postmortem examination of brains of patients who have died from COVID-19 shows evidence of neuroinflammation, mainly consisting of activated microglia.[19–21] This is also seen by a preclinical postmortem study in primates with COVID-19.[22] Involvement of the brain in the generalised inflammatory response following SARS-CoV-2 infection is in line with studies with [^{18}F]Fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography (PET), as several studies reveal patterns of reduced glucose metabolism in the brain, which is a reflection of decreased neural metabolism.[23–26] (Neuro)inflammation can also directly be investigated in vivo with [^{18}F] *N,N*-diethyl-2-(2-(4-(2-[18F]fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a] pyrimidin-3-yl)acetamide (DPA-714) PET.[27] [^{18}F]DPA-714 binds with high affinity to the 18 kDa translocator protein (TSPO), which is mainly expressed on activated macrophages, astrocytes and microglia.[28 29] Other studies demonstrated that TSPO PET is able to quantify (neuro) inflammation in multiple sclerosis, Alzheimer's disease, rheumatoid arthritis[29–31] and chronic fatigue.[32] Involvement of the brain has also been shown by longitudinal MRI studies in subjects with SARS-CoV-2 infection where modest structural and microstructural differences were seen compared with subjects who were not infected.[33–36] Moreover, research with (functional) MRI (fMRI) of the brain in other groups of patients with persistent fatigue and cognitive complaints, such as myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and multiple sclerosis, have shown grey matter abnormalities,[37 38] reduced functional connectivity[39–41] and altered neuronal activity during cognitive tasks.[42]

The VeCosCO study consists of two work packages. The objectives of work package 1 (WP-1) are (1) investigate the relation between persisting fatigue and concentration problems and neuropsychological functioning; (2) determine risk factors and at-risk phenotypes for the development of persistent fatigue and cognitive complaints, and the presence of PEM and (3) describe consequences of persistent complaints on quality of life, healthcare use and physical functioning. The objectives of WP-2 are to (1) determine the presence of neuroinflammation with [^{18}F]DPA-714 whole-body PET scans and (2) explore the relationship between (neuro)inflammation and brain structure and functioning measured with MRI.

METHODS

Design

This is a case–control study in participants with laboratory-confirmed SARS-CoV-2 infection. Individuals with persistent fatigue and concentration problems and individuals without these persistent complaints >3 months after SARS-CoV-2 infection will be compared. Both groups will consist of individuals who have been admitted to the hospital and individuals who stayed at home during the acute phase of the illness.

Study population

The inclusion and exclusion criteria for both work packages can be found in table 1. For WP-1, 200 individuals will be recruited, of which n=122 with persistent fatigue and concentration problems and n=78 people without these persistent complaints following COVID-19. WP-2 will recruit a subgroup of 55 participants from WP-1 of whom 40 patients have persistent complaints, aged between 30 and 65 years, and 15 patients without persistent complaints with similar age sex and hospital distribution. The majority of participants are recruited from existing COVID-19 cohorts in the Netherlands: the RECoVERED,[1] NeNeSCo,[43] ReCOVer,[44] LongCOVID[45] studies and a cohort of infected healthcare workers (HCWs) from the University Medical Centers (UMC) Utrecht. All participants were initially infected with SARS-CoV-2 between March 2020 and December 2021, with the exception of the LongCOVID study in which inclusion is ongoing. The HCW cohort concerns employees took part in an online questionnaire study assessing time to return to work and persisting symptoms. In addition to the cohort studies, individuals will be included from the post-COVID-19 outpatient clinic of the Amsterdam

Table 1 Inclusion and exclusion criteria

Inclusion criteria work package 1		Exclusion criteria work package 1
a. At least 3 months after diagnosis of COVID-19 (hospitalised or non-hospitalised) b. COVID-19 confirmed by a positive PCR for SARS-CoV-2, positive SARS-CoV-2 serology or CO-RADS (COVID-19 Reporting and Data System) ≥4 on CT-scan, or antigen rapid test		a. Known psychiatric or somatic condition that could explain the current fatigue or cognitive symptoms. b. Severe fatigue or cognitive complaints prior to COVID-19. c. Insufficient command of the Dutch language. d. Re-infection with SARS-CoV-2 within 3 months. e. A score ≥35 on the CIS fatigue scale but a score <18 on the CIS concentration scale OR a score <35 on the CIS fatigue scale but a score ≥18 on the CIS concentration scale
With complaints*	**Without complaints***	
a. A score ≥35 on the CIS fatigue scale AND b. A score ≥18 on the CIS concentration scale	a. A score <35 on the CIS fatigue scale AND b. A score <18 on the CIS concentration scale	
Inclusion criteria work package 2		**Exclusion criteria work package 2**
a. 30–65 years of age b. rs6971 genotyping shows mixed or high affinity binding		a. Rs6971 shows low affinity binding b. Haemoglobin ≤8 (males) or ≤7 (females) c. Being unable to lay still for scanning due to claustrophobia, severe back pain or trypanophobia (fear of needles) d. Gross neurological pathology (strategic or lobar infarcts or stroke or neurotrauma) on MRI or CT that may interfere with the interpretation of the PET scan e. Females of childbearing potential who are not surgically sterile, not refraining from sexual activity or not using reliable methods of contraception. Females of childbearing potential must not be pregnant or breastfeeding at screening f. Having donated blood within 6 months prior to the PET scan day g. Current use of benzodiazepines
With complaints	**Without complaints**	
a. Severe fatigue on the CIS (≥35 on the fatigue scale) b. Concentration problems on the CIS (≥18 on the concentration scale) c. Physical/social disability (≤65 on the RAND-36 physical functioning subscale or a score of ≥10 on the WSAS)	a. No severe fatigue on the CIS (<35 on the fatigue scale) b. No concentration problems (<18 on the concentration scale) c. No physical/social disability (>65 on the RAND-36 physical functioning subscale or a score of <10 on the WSAS)	

*Complaints consist of persistent severe fatigue and concentration problems.
CIS, Checklist of Individual Strength; PET, positron emission tomography; RAND-36, Research and Development-36; WSAS, Work and Social Adjustment Scale.

UMC (AUMC). If recruitment from these cohorts is not sufficient, participants will be recruited from outside the cohorts.

Interested participants from existing cohorts are contacted to discuss potential enrolment. After obtaining written informed consent, the checklist individual strength (CIS) will be sent to verify eligibility to participate. Consent will be collected for the sharing of original cohort data with the VeCosCO study. Participants for WP-2 are recruited from participants of WP-1 with additional screening of inclusion and exclusion criteria. RS6971 polymorphism will be determined as TSPO PET binding is influenced by genotype. Low affinity binders will be excluded for WP-2.[46]

Study procedure

A flow chart of the study procedure can be found in figure 1. WP-1 participants will have a single study visit, during which participants undergo a neuropsychological assessment and several physical tests. Furthermore, a venous puncture will be done to collect 10 mL whole blood in EDTA tubes, which will partly be stored whole blood/buffy coat (−20°C or −80°C), and used for DNA isolation for RS6971 polymorphism and ApoE-E4 genotyping. Prior to the study visit, participants are asked to complete a battery of validated web-based questionnaires. After 9 months participants are asked to complete a subset of the web-based questionnaires again. The Fatigue and Energy Scale (FES) is administered directly before and after the study visit to assess PEM following the study visit.

A subset of 40 participants from WP-1, 20 with and 20 without persistent fatigue, concentration problems and PEM will be included in a substudy on PEM. Participants are asked to wear an actigraph during 1 week before and 1 week after the study visit to measure their level of physical activity objectively. Additionally, they will complete an Ecological Momentary Assessment (EMA) measure

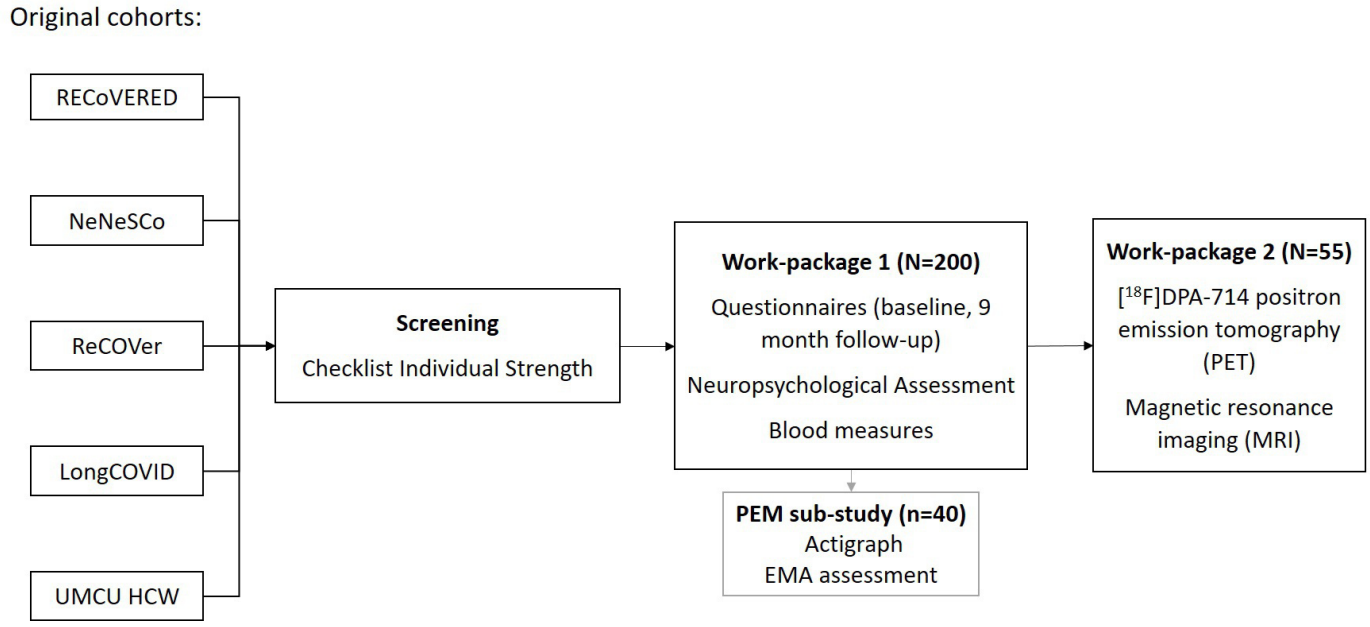

Figure 1 Flow chart of the study procedure. EMA, Ecological Momentary Assessment; HCW, healthcare worker; PEM, postexertional malaise; UMCU, University Medical Center Utrecht.

of symptoms and activity during the 14 days. The FES is administered 1 week before and 1 week after the visit.

A subset of 55 participants from WP-1 will be included in WP-2 aiming to quantify in vivo (neuro)inflammation using [^{18}F]DPA-714 PET. Dynamic PET scans (70 min) will be acquired on a PET/CT or total body PET/CT, alternately capturing brain (60 min) and body (depending on scanner type, with a maximum of 30 min; pelvic to head) with both continuous on-line and manual arterial blood sampling for full quantification (ie, non displaceable binding potential). Additionally, structural and fMRI scans (T1/MPRAGE, FLAIR, resting state fMRI, T2-weighted images (WI) and multishell DTI) will be acquired in all participants.

Outcomes

An alphabetised list of all questionnaires and questionnaire information in the VeCosCO protocol can be found in online supplemental 1. We sometimes use multiple instruments per dimension to allow for comparison with data previously collected in the cohorts, which used different instruments.

Screening measures

To determine the presence of severe fatigue and concentration problems, the CIS subscales fatigue and concentration are used.[47] The fatigue subscale has a validated cut-off of ≥35. For the concentration subscale, a cut-off threshold of ≥18 defines the presence of notable concentration problems. This cut-off is based on data from Worm-Smeitink *et al* a study in 1923 healthy subjects who completed the CIS, and corresponds with 85% of healthy subjects scoring below this threshold.[48]

For the PEM substudy, 20 participants with complaints who report PEM and 20 participants without complaints and PEM are included. The presence of PEM is determined with a question formulated by the Centers for Disease Control and Prevention as a criterion for ME/CFS.[49] The frequency of PEM is assessed using a four-point Likert scale: (0) not at all, (1) a few times a month, (2) a few times a week and (3) every day. Duration was assessed with a three-point scale: (0) not, (1) less than 6 months and (2) longer than 6 months. Presence of this complaint at least a few times a month, for more than 6 months defines PEM.

Neuropsychological functioning

The Montreal Cognitive Assessment (MoCA) is used to assess general cognitive functioning. In addition, extensive neuropsychological assessment will be done for the following cognitive domains:

Performance validity is measured with the Test of Memory Malingering (TOMM). The TOMM is a visual learning test that is used to detect performance validity, as it is insensitive to true memory or learning impairments. Attention is measured with Stroop parts 1 and 2, Trail Making Test (TMT) part A, D2 test and Digit-span forward. Executive functioning is measured with the Digit-span backwards, Stroop Color-Word Test, the Controlled Oral Word Association Test and TMT-B. Memory will be assessed with the Dutch translation of the Rey Auditory Verbal Learning test and the recall condition of the Rey Complex Figure test. Visuoconstruction will be assessed with the copy condition of the Rey Complex Figure test. Language is assessed through the animal fluency test.

PEM substudy

An actigraph is used to assess the participant's level of physical activity. The actigraph is worn around the wrist for 14 consecutive days and nights for an estimate of

daily activity. The actigraph has been shown to be a reliable and valid instrument for the assessment of physical activity.[50] Using EMA, participants are asked to report their current level of fatigue, concentration problems, intensity of social/mental activity and the presence of headaches and/or muscle pain.[51] Participants will receive five text messages during daytime from 1 week prior to 1 week after the study visit.

[^{18}F]DPA-714 PET (only measured in WP-2)

Whole-body and neuroinflammation will be investigated using PET with [^{18}F]DPA-714. More specifically, following a low-dose CT for attenuation correction, an emission scan will be acquired after a bolus injection of approximately (mean) 260 (±circa 10%) MBq [^{18}F]DPA-714. The scanners used are the Biograph Vision Quadra from Siemens Healthineers and the CT 5000 Ingenuity CT Scanner from Philips. Arterial blood will be sampled continuously at a rate of 300 mL/hour for the initial 5 min, and 150 mL/hour in the 55 min thereafter, while using an online detection system. In addition, manual blood sampling will be performed during the scans on fixed timepoints (T=5, 10, 15, 20, 40, 50, 60, 75 and 90 min post injection) of approximately 8 mL, which will be used to estimate plasma-to-whole blood ratios and to measure plasma metabolite fractions. A detailed description of the radiometabolite analyses has been published previously.[31] Dynamic PET acquisition will be performed in list mode, with default reconstruction protocols, including all usual corrections, for example, for attenuation, scatter, randoms, decay and dead time. Image preprocessing has been described elsewhere.[29 52] In brief, for brain tissue segmentation, 3D T1-weighted structural MRI scans (MPRAGE sequence) will be acquired. For image analyses/quantification, structural 3D T1-weighted MRI images (brain) and CT images (body) will be co-registered and superimposed to the PET images. Subsequently, for brain image analyses, PVElab will be used to derive time activity curves in anatomically based regions of interest on a probability atlas of the human brain.[53] Based on earlier findings, a reversible two-tissue compartment model with additional blood volume parameter will be used for [^{18}F]DPA714 quantification,[29] and valid parametric methods will be further investigated and applied for voxel-wise comparisons (eg, Logan plot analysis).

To exclude potential effects of the COVID-19 pandemic, we will additionally use five [^{18}F]DPA-714 PET scans of healthy controls (prepandemic historical data, mean age 52y, 43% male).

MRI (only measured in WP-2)

Brain MRI will be performed on a 3 Tesla operating MR scanner to obtain structural and functional information on the brain. Several MRI sequences (FLAIR, DTI, T2-WI and T1/MPRAGE) will be used to be able to visualise and quantify structural brain damage as a result of inflammation and prolonged inflammatory responses. Resting-state fMRI (EPI) will be acquired to determine functional brain network characteristics.

Briefly, functional images will be preprocessed for analyses with default settings (motion-corrected, time-filtered), non-linearly spatially normalised, resampled and smoothed. In addition, a regression of confounds will be performed to account for slow time drifts, high frequencies, motion parameters, average signal of the white matter and the ventricles. Structural MRI data will be preprocessed with Freesurfer using a standardised pipeline, and through SPM12 for voxel-wise comparison (after spatial normalisation).

Other outcome measures

The Fatigue Severity Scale is a self-administered questionnaire investigating the severity of fatigue in different situations during the past week. The FES is used to assess postexertional exacerbation of fatigue. As no Dutch version of the FES existed, we translated the questionnaire and a backwards translation validation was done by the authors of the original version.

The Cognitive Failure Questionnaire (CFQ) is a subjective cognitive functioning questionnaire about everyday cognitive failures. The Checklist for post-IC Cognitive Complaints (CLC-IC) is adapted from the Checklist of Cognition and Emotion (CLC-24)[54] and is used to identify cognitive problems after being hospitalised on the Intensive Care Unit (ICU).

Healthcare consumption and productivity loss are assessed using the adapted version of the Treatment Inventory of Costs in Patients with psychiatric disorders (TIC-P). Self-reported generic health status is assessed by the Short Form Health Survey 36 (SF-36) in WP-1 and the Research and Development-36 in WP-2.

Orthostatic intolerance is measured with the Composite Autonomic Scoring Scale subscale. The presence of common symptoms associated with chronic fatigue is assessed by the DePaul Symptom Questionnaire SF.

Skeletal muscle function will be measured with the Medical Research Council Sum Score,[55] lower extremity function will be assessed by measuring walking speed, balance and leg strength with the Short Physical Performance Battery.[56] Endurance will be measured with the 2 min step test (TMST) which is part of the Functional Fitness Test.[57] During the TMST, blood pressure, heart-rate and oxygen saturation will be measured using a blood pressure monitor and pulse-oximeter.

Predictor variables

Sociodemographic factors age, gender, birth country and educational level will be collected.

Clinical factors such as hospital and ICU admission (yes/no), date of diagnosis and hospital discharge, comorbidities and vaccination status will be obtained from the cohort databases.

Responses to symptoms are assessed by the 16-item Cognitive and Behavioural Responses to Symptoms Questionnaire.[58] Illness perceptions are assessed using the

Brief Illness Perceptions Questionnaire.[59 60] Self-efficacy concerning fatigue is measured with the Self-Efficacy Scale 28.[61] Catastrophising of fatigue is assessed with the Jacobson-Fatigue Catastrophising Scale.[62] Sleep problems are assessed using the Insomnia Severity Index[63] and Pittsburgh Sleep Questionnaire.[64] The presence of depressive symptoms is determined using the depression subscale of the Hospital Anxiety and Depression Scale[65] and the Patient Health Questionnaire-9.[66]

Sample size calculation

For WP-1, the primary outcome measure is neuropsychological functioning. A previous study among 58 patients 2–3 months after the onset of moderate or severe COVID-19 infection and 30 appropriately matched uninfected controls found a median MOCA score of 27 (IQR 25–29) among cases and a median score of 28 (IQR 27–29) among controls.[67] This would correspond with a mean score of 27 (SD 3.041) and 28 (1.56) in cases and controls, respectively, and a Cohen's d effect size of 0.64.[68–70] Because this study will compare participants with persistent complaints after COVID-19 with participants without persistent complaints after COVID-19, that is, not with healthy controls, we expect a smaller effect size. Assuming a difference between participants with and without persistent complaints on the MOCA score of a medium sized magnitude (Cohen's d=0.5), a power of 0.80 and a two-sided p value of 0.025 (corrected for multiple tests) a sample size of 78 per group would be sufficient. Additionally, we aim to investigate risk factors for persistent complaints. We will investigate the predictive value of a total of 20 potential sociodemographic, illness-related, cognitive-behavioural and psychosocial risk factors. According to the rule of thumb to have at least n=10 per predictor variable, a total of 200 participants will be included in WP-1.

We assume that the level of symptoms increase and activity levels decrease more after exertion (ie, the WP-1 study visit) among participants with persistent complaints than among participants without persistent complaints. To be able to detect a difference in slopes between the groups with and without complaints and PEM (repeated measures analysis of variance, within-between interactions) with a medium sized effect (f=0.25), 80% power, a two-sided alpha of 0.05, with 70 measurements and a correlation of 0.4 among repeated measurements, a total of at least 20 participants would be required for the PEM substudy.

For WP-2, since, to the best of our knowledge, this is the first study using [^{18}F]DPA-714 PET (or a comparable) tracer in a population of COVID-19 patients, the expected effect size are lacking. Hence, the number of patients is based on a proof of concept PET study with an earlier generation PET tracer for neuroinflammation in subjects with CFS compared with controls.[32] Based on PET binding, eight participants per group was sufficient to account for differences between groups and to detect statistical power (0.80, alpha 0.05). Given desired power of 0.9, we will use 40 patients and include 15 controls.

Patient and public involvement

The Dutch Long COVID patient association was involved in the setup of this study. C-support, a Dutch association that represents the interests of COVID-19 patients, will be involved in the interpretation and dissemination of the research.

DATA HANDLING AND ANALYSIS

The handling of data will comply with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation. Data will be electronically stored in Castor EDC with the exception of imaging data and bodily material. Access will only be provided to authorised staff. Each patient will receive a personal identification number, data will therefore be stored pseudonymously. The data dictionary will be exported every time the eCRF is updated.

Cohort data will be shared in read-only files and re-entered in the eCRF. Collected data will be entered in Castor EDC.[71] After inclusion is completed and all assessments are done, the Castor EDC database will be locked. Data (paper and electronic) will be kept in storage for 15 years. Bodily material (ie, blood) will be handled using the coded subject identification number and (temporarily) stored at the central laboratory of the AUMC. When the ApoE genotype and TSPO genotype have been determined, the blood samples will be destroyed. Imaging data (MRI and PET) will be acquired and stored under participants identification number, on a secure hospital network that is only accessible on permission.

Statistical analysis plan

We will investigate if there are differences in neuropsychological functioning between patients with and without persistent fatigue and concentration problems using logistic regression, adjusted for time since infection, sex, age and education level. Additional post hoc analysis will be done to investigate whether infection at home versus hospital admission, as a proxy for severity of initial infection, moderated effects. We will use logistic regression to investigate whether specific predictors (age, gender, educational level, hospitalisation, cognitive functioning, coping and inflammation/genotype markers) are associated with persisting severe fatigue and concentration problems after COVID-19. Additionally, we will use principal component analysis to identify subtypes and latent factors in relation to persistent complaints across all participants. Differences in quality of life, healthcare use and physical functioning between individuals with and without persistent complaints will be analysed using t-tests and χ^2 tests. These statistical analyses will be performed using Stata (V.15.1) and IBM SPSS statistics (V.28).

The presence of PEM will be analysed using linear mixed models for the EMA measurements at 70 time

points. It will be determined if symptoms increase and activity levels decrease more among participants with persistent complaints compared with participants without persistent complaints following the WP-1 study visit, which is assumed to lead to PEM.

To compare systemic and neuroinflammation measured with [^{18}F]DPA-714 between groups (persistent and no persistent complaints and historic controls), we will perform linear regression analyses on regional and global (brain) regions, with and without adjustments for common confounding effects (eg, age, sex, TSPO genotype). We will repeat analyses on a voxel-wise level using parametric DPA714 images in Statistical Parametric Mapping V.12 (SPM12). To investigate whole-body inflammation, we will use in house developed software to investigate standardised uptake values and target-to-background ratios in regions of interest.[72] Additionally, we will investigate differences between groups on structural and fMRI outcome measures in a priori defined (brain) regions as well as large scale brain networks (for fMRI) in MatLab and SPM12.[33 73]

ETHICS AND DISSEMINATION

WP1 (NL79575.018.21) and 2 (NL77033.029.21) were approved by the medical ethical review board of the AUMC. All participants will provide a written informed consent.

This study is subject to on-site monitoring in accordance with the quality assurance advice of the Dutch Federation of University Medical Centres regarding research involving human subjects.[74] On-site monitoring is based on the risk classification negligible. The investigator will submit a summary of the progress of the trial to the ethics committee once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included, and amendments.

Individual results of the study are not shared, but participants can request their data. If haemoglobin levels, cognitive functioning or MRI are found to be abnormal, the participant and general practitioner will be notified. The TSPO result will be shared if the patient is eligible for inclusion in WP-2 or on request.

Data transfer agreements have been signed for the sharing of data collected in the original cohorts with the VeCosCO researchers.

Results of this study will be submitted for publication in peer-reviewed journals and shared with the key population, individuals with persistent complaints after COVID-19.

DISCUSSION

The VeCosCO study, consisting of two work packages, aims to investigate the relation between persisting complaints and neuropsychological functioning, risk factors and at-risk phenotypes for the development of persistent fatigue and concentration problems, including the presence of PEM, and consequences of persistent complaints on quality of life, healthcare use and physical functioning. In addition, WP-2 aims to determine the presence of neuroinflammation in patients with persistent fatigue and concentration problems after COVID-19, and explore the relationship between (neuro)inflammation and brain structure and functioning measured with MRI. This research will add to the limited knowledge on the mechanisms that may lead to long COVID.

There are three deviations from the protocol published in a preprint.[75] First, participants with only a single complaint (ie, fatigue or concentration problems) will be excluded from WP-1 and only participants expressing both complaints will be included to create the highest possible contrast between the groups with and without persistent complaints. As a result, inclusion criteria for WP-1 and WP-2 are not equal. Second, due to a switch to a more sensitive PET scanner with lower radiation burden, the age range for inclusion in WP-2 is lowered to 30. Additionally, the upper age range is expanded to 65 years old. Third, it was decided to include only individuals with high affinity TSPO binding to eliminate potential differences in binding of the radioligand due to underlying genotype.

STUDY STATUS

Patient recruitment started in May 2022. At the time of revised submission, 208 participants have been included in WP-1 and 43 in WP-2. Completion of inclusions for WP-1 is expected in July 2023. WP-2 is expected to be complete in September 2023.

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Acknowledgements The authors wish to thank all VeCosCO study participants. In addition, we wish to thank all members of the VeCosCO Study Group, listed below, and original cohorts.VeCosCo Study Group: Indiana Buenting, Eva Camper, Annelotte Kooij, Roos Rikken, Muska Uriakhel, Anne Visser.

Contributors Conceptualisation, BNMvB, NT, HK and SCJV; Funding acquisition, BNMvB, NT, HK and SCJV; Investigation, AJCS, AV, SCJV, DV, DWK, EMvdG, EMC, FB, HEH, IC, JMAV-M, RB, SG and YMGvO; Participant inclusion, BA, GdB, CMvH, JH, MDdJ, TAK, TvdM, YMGvO, MP, MvV and CCvdW; Project administration, AV and DV; Resources, BNMvB, HK, NT and SCJV; Supervision, BNMvB, HK, NT and PTN; Writing—original draft, AV, DWK, IC and SCJV; Writing—review and editing, all authors.

Funding This publication is part of the project 'VeCosCO: Neurobiologische basis van langdurige cognitieve klachten en vermoeidheid na COVID-19' with project number 10430302110003 of the research programme COVID-19, financed by the Netherlands Organisation for Health Research and Development (ZonMw). HK received additional funding from 'ReCOVer: A Randomised Controlled Trial testing the efficacy of Cognitive Behavioural Therapy for preventing chronic postinfectious fatigue among patients diagnosed with COVID-19' with project number 10430012010025 of the research programme COVID-19, financed by ZonMw. PTN and AV received additional funding from 'Long-term mental health trajectories in recovered COVID-19 patients: exploring the interplay of psychosocial and biological factors affecting health-related quality of life' with project number 10430032010010 of the research programme COVID-19, financed by ZonMw. FB is supported by the NIHR biomedical research centre at UCLH

Competing interests FB: Steering committee or iDMC member for Biogen, Merck, Roche, EISAI and Prothena. Consultant for Roche, Biogen, Merck, IXICO, Jansen, Combinostics. Research agreements with Merck, Biogen, GE Healthcare, Roche. Co-founder and shareholder of Queen Square Analytics.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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