NeuroCOVID-19: a longitudinal protocol for observing impact on brain structure and function in individuals recovering from COVID-19 after hospitalization or self-isolation

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Keywords:	Neurology, Radiology and imaging, Infectious diseases, Behavioural sciences
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Abstract:	Background: NeuroCOVID-19, a longitudinal observational study, was initiated in April 2020 to characterize the potential impact and lingering effects of coronavirus disease 2019 (COVID-19) on the human brain, among individuals who experience a broad range of COVID-19 illness. The protocol involves collecting imaging and behavioural data for parallel lines of investigation and integrated analyses. Methods: NeuroCOVID-19 initially aims to recruit 180 adults into three groups: individuals who are no longer infectious after self-isolation or hospitalization due to COVID-19, and controls who are no longer

	infectious after self-isolation due to flu-like symptoms and test negative for COVID-19. Initial and 3-month follow-up assessments include brain magnetic resonance imaging (MRI), behavioural assessment (sensation, cognition, mood and symptom self-report), and electroencephalography. Lung MRI is also included to study how brain changes relate to pulmonary changes. Informed by the other measures, the primary aim is to test for group differences in brain lesions assessed by high-resolution anatomical MRI. Regional cerebral blood flow, diffusion, resting state, and Gadolinium-contrast enhanced MRI data are also acquired. Results: Twenty-seven COVID-19 participants (25 self-isolated, 4 hospitalized) and 11 controls have completed initial assessment with approximately half receiving follow-up. Recruitment is on-going. Alterations in brain anatomy are evident in both the self-isolated and hospitalized COVID-19 groups, spanning young adult-hood to advanced age. Interpretation: The NeuroCOVID-19 protocol consists of anatomical, physiological, symptomological, and behavioural phenotyping for deep characterization of how COVID-19 may impact the brain, potentially with lingering effect.

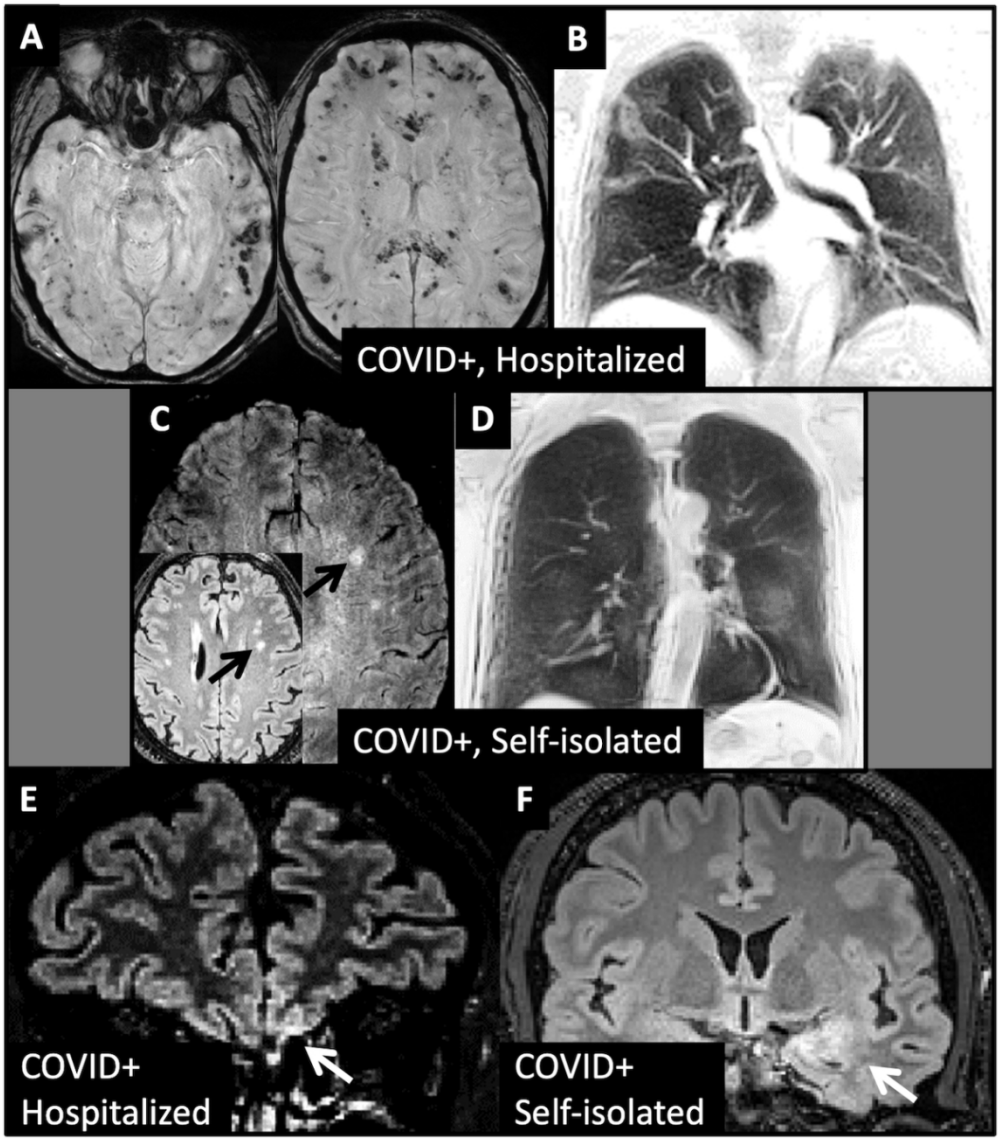

Figure 1. Brain and lung images from a selection of study participants. Top: a COVID+ male in his 50's who spent >12 days on a ventilator before recovering showed (A) numerous moderate sized lesions on SWI and (B) substantial abnormal signal intensities on lung imaging. This individual had severely impaired olfaction and moderate behavioural impairment (NIH toolbox 17th percentile on working memory, and complete inability to discriminate previously seen items from similar lures). He has been referred to a neurology clinic for follow-up. Middle: a COVID+ male in their 50's who self-isolated and showed micro-lesions in (C) SWI and T2 FLAIR imaging (inset), with (D) corresponding lung image with diffuse changes. Olfaction and sensory/behavioural tests were within age norms but self-report indicated difficulty in performing activities of daily living involving memory. Bottom: a COVID+ female in their 50's with moderately impaired olfaction and (E) elevated T2-FLAIR signal near the olfactory bulb and orbitofrontal cortex; and a COVID+ male in their 30's with an encephalitis-like lesion in the temporal lobe as observed (F) on T2-FLAIR imaging.

101x116mm (300 x 300 DPI)

NeuroCOVID-19: a longitudinal protocol for observing impact on brain structure and function in individuals recovering from COVID-19 after hospitalization or self-isolation

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Abstract

Background: NeuroCOVID-19, a longitudinal observational study, was initiated in April 2020 to characterize the potential impact and lingering effects of coronavirus disease 2019 (COVID-19) on the human brain, among individuals who experience a broad range of COVID-19 illness. The protocol involves collecting imaging and behavioural data for parallel lines of investigation and integrated analyses.

Methods: NeuroCOVID-19 initially aims to recruit 180 adults into three groups: individuals who are no longer infectious after self-isolation or hospitalization due to COVID-19, and controls who are no longer infectious after self-isolation due to flu-like symptoms and test negative for COVID-19. Initial and 3-month follow-up assessments include brain magnetic resonance imaging (MRI), behavioural assessment (sensation, cognition, mood and symptom self-report), and electroencephalography. Lung MRI is also included to study how brain changes relate to pulmonary changes. Informed by the other measures, the primary aim is to test for group differences in brain lesions assessed by high-resolution anatomical MRI. Regional cerebral blood flow, diffusion, resting state, and gadolinium-contrast enhanced MRI data are also acquired.

Results: Twenty-seven COVID-19 participants (25 self-isolated, 4 hospitalized) and 11 controls have completed initial assessment with approximately 50% completing follow-up. Recruitment is on-going. Alterations in brain anatomy are evident in both the self-isolated and hospitalized COVID-19 groups, spanning young adult-hood to advanced age.

Interpretation: The NeuroCOVID-19 protocol consists of anatomical, physiological, symptomological, and behavioural phenotyping for deep characterization of how COVID-19 may impact the brain, potentially with lingering effect.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its related illness coronavirus disease 2019 (COVID-19) can impact not only the lungs, but other organs including the brain. Focusing on acute presentations of hospitalized patients, recent reviews have discussed neuroinflammatory and vascular mechanisms of brain injury due to COVID-19 (1,2), whereas other research suggests that COVID-19 especially increases risk of large vessel stroke and multi-territory infarcts (3,4). Ischemic stroke may be due to SARS-CoV-2 depleting bioavailability of the angiotensin-converting enzyme-2 (ACE2) receptor, whereas hemorrhagic stroke points to vulnerability of the endothelial cells to the coronavirus (5,6).

Little is known about mid- and long-term neurocognitive outcomes across the spectrum of COVID-19 illnesses, despite the recognition that acute symptoms may persist after hospital discharge (7,8) and that "long-haul" symptoms include a neurological component (9). Moreover, neurological effects of COVID-19 are likely under-reported at present in the scientific literature, which focuses primarily on hospitalized patients - not the much larger number of individuals who were triaged or otherwise self-isolated, some with concerning symptoms. To address these issues, we have thus developed a novel experimental protocol (NeuroCOVID-19) to characterize the broad-ranging brain changes due to COVID-19, and their time-course. The protocol includes: a) high-resolution anatomical magnetic resonance imaging (MRI) to visualize gross neuropathology as well as subtle effects in individuals, such as small vessel disease, narrowing of the olfactory cleft, or potential impact on the olfactory bulb or medulla oblongata from coronavirus invasion of the olfactory or trigeminal nerves (10); b) MRI of physiological function to assess impact (at the group level) on cerebral hemodynamics, blood brain barrier (BBB) permeability, neuroinflammation, white matter microstructure, and resting state connectivity (11); c) state-of-the-art breath-hold lung MRI to assess neural-respiratory relationships; and d) characterization of deficits in smell, vision, domain-specific cognitive impairment, mood, and electroencephalography (EEG) measures, as appropriate to document longitudinal brain/behavioural characteristics and symptoms. The primary hypothesis is that at initial assessment (when no longer

infectious), individuals with COVID-19 have increased brain lesion burden relative to a control group of individuals who had flu-like symptoms but tested negative for COVID-19. Second, COVID-19 is hypothesized to yield neurophysiological, olfactory, and behavioural alterations detected at initial and 3-month follow-up assessments. Exploratory analyses are also planned to identify potential at-risk profiles among COVID-19 individuals by investigating associations between brain measures, lung measures, symptoms and their duration, cognitive and behavioural deficits, and EEG measures.

Methods:

Study design

NeuroCOVID-19 is a longitudinal observational study that is recruiting non-infectious participants in three cohorts: individuals who contracted COVID-19 and were either 1) hospitalized, or 2) self-isolated; and age- and sex-matched controls, 3) with recent flu-like symptoms who tested negative for COVID-19 and were self-isolated. The target sample size for each cohort is 60 (30 males, 30 females). Brain imaging, sensory, self-report, and cognitive assessments are administered at initial and 3-month follow-up hospital visits, interleaved between 3 virtual visits (Table 1).

Setting

The protocol and informed consent form were approved by the Research Ethics Board and Infection and Prevention Control at Sunnybrook Health Sciences Centre on April 4, 2020. Recruitment is ongoing. Participants provide free and informed consent prior to study initiation, and all in-hospital assessments are administered while research staff and participants wear personal protective equipment.

Participants

Participants are eligible for inclusion if they are from 19-75 years old, have documented proof of their COVID-19 test result and reside in the community. Exclusion criteria include previous dementia,

neurological disorder, severe psychiatric illness, traumatic brain injury, on-going unstable cardiovascular disease, and contraindications to MRI (e.g., ferromagnetic implants). Contrast-enhanced MRI (see below) is not performed in individuals with poor kidney function (est. glomerular filtration rate < 60 mL/min/1.73 m^2), or at follow-up. Recruitment is undertaken through the emergency department electronic database, physician referrals, and advertisements in the community and on social media.

Data sources

COVID-19 status. Diagnosis is determined by a provincial-approved facility through a nasopharyngeal or oropharyngeal swab and subsequent real-time reverse transcription polymerase chain reaction (RT-PCR) test, according to Public Health Ontario procedures (12).

3 Tesla MRI. The MRI protocol (Table 2) includes high-resolution anatomical imaging (T1-weighted, T2-weighted, fluid-attenuated inversion recovery, and susceptibility weighted imaging) to identify brain lesions and permit tissue density and volumetric analysis. Pseudo-continuous arterial spin labeling (pCASL) MRI is performed using a single post-labeling delay to quantify cerebral blood flow (CBF). Resting state functional MRI (rs-fMRI) is performed to evaluate regional brain connectivity. Triple-shell diffusion weighted imaging (DWI) is performed to probe microstructural integrity of the white and grey matter using multiple metrics. Lung MRI is performed with an ultra-short echo time rapid breath-hold acquisition. Dynamic Susceptibility Contrast (DSC) MRI is performed using a common MRI contrast agent (Gadovist, Bayer; injected at a dose of 1 ml/kg with 25cc saline flush at a rate of 5cc/sec) to evaluate cerebral hemodynamics. Ten minutes post-injection, T1-weighted MRI is repeated to visualize potential BBB leakage.

After a short break, including water and/or snack, participants complete the following tests in a soundproof assessment room.

Smell. The 40-odorant University of Pennsylvania Smell Identification Test (UPSIT, Sensonics International) characterizes olfactory function relative to normed data, yielding results ranging from normosmia to severe/total anosmia. The UPSIT is well established for assessing olfactory dysfunction from COVID-19 (13).

Vision: As COVID19 may have possible impacts on the eye (14) or neural visual perceptual functions, the Freiburg Vision Test (FrACT) (15) is used to measure far visual acuity and vernier acuity, and binocular contrast sensitivity is measured using the Pelli-Robson Contrast Sensitivity Test.

Cognition. The NIH Toolbox full Cognition Battery is administered using an iPad app (16), covering perceptual, attention, executive, language, and memory domains. A Mnemonic Similarity Task test is also included to supplement memory assessment, sensitive to age-related memory decline, as well as hippocampal function and connectivity (17).

Mood & Functional Outcome: Emotional status is assessed using the full NIH Emotion toolbox administered remotely. Subjective cognitive, sleep, fatigue, dyspnea and general functional status are collected using the PROMIS tools (18).

Electroencephalography (EEG): A four-channel wireless EEG headband (Muse, InteraXon, Toronto, CA, RRID:SCR_014418) is used to record EEG at rest with eyes closed (5 min) and eyes open (10 min) (19), during an auditory oddball task in which participants respond to infrequent target tones and ignore frequent standard tones, and during a visual perceptual task that requires discriminating a contour in a cluttered background of increasing density (20). Spectral EEG power and event-related potentials will be used to probe possible changes in neural function (21), brainstem dysfunction associated with cognitive impairment, and delays in visual perceptual processes.

Electroencephalography (EEG): A four-channel wireless EEG headband (Muse, InteraXon, Toronto, CA) is used to record EEG at rest with eyes closed (5 min) and eyes open (10 min) to assess possible disruptions in mental processing (21); as well as during an oddball task (P300) in which participants respond to infrequent target tones and ignore frequent standard tones, and a clutter test that requires discriminating a contour in a cluttered background of increasing density. These tasks probe possible brainstem dysfunction associated with cognitive impairment (19,20).

Primary outcome

Lesion volume burden is the primary outcome and is based on the aggregate of white matter hyperintensities (WMH), microbleeds, infarcts and areas of inflammation, which are hypothesized to occur more frequently in COVID-19 survivors than controls. Importantly, MRI-visible vascular lesions are related to aging and vascular risk factors, and over their lifetimes. In addition, COVID-19 negative control participants may have had exposure to other potential pathogens (e.g. bacterial or viral) that are capable of CNS invasion (22). A linear regression model will be used to test for an association between brain lesions and COVID-19 while accounting for age and sex as covariates. Days spent in the intensive care unit and the cardiovascular disease Framingham Risk Score (23) will be added to the model in separate sensitivity analyses. A preliminary power analysis using G*Power software estimates that for N=180 cross-sectional samples, alpha=0.01, and power=0.80, an effect size of 0.28 is required to show a significant parameter estimate that relates a normally distributed measure of brain lesion volume with the COVID-19 group (critical F=4.73, df1=2,df2=175).

Data Analysis

Anatomical brain and lung MRI data will be scored by research neuroradiologists. The high spatial resolution brain images (1 mm isotropic voxels) will also permit detailed quantification of brain lesion burden using semi-automatic Sunnybrook software (24), as required for primary analysis. The lung images will be scored by adapting a scale used to assess lung computed tomography (CT) findings in COVID-19 patients (25). Additional secondary analyses will be conducted using established neuroimaging freeware packages for pre-processing and calculating output parameters. For example, DWI data will be analyzed to account for free water using a two-compartment model (26) using DIPY (27). DSC images will be processed to estimate CBF, cerebral blood volume (CBV), mean transit time, and contrast leakage maps using 3D Slicer software (28). Standard analyses for neurophysiological, sensory, and cognitive data will also be performed (e.g. according to NIH toolbox procedures). Univariate model testing will be conducted to assess whether the effects of COVID-19 on individual outcome variables persist from initial testing to 3-months follow-up, relative to controls. Finally, linear mixed effects models, and data-driven multivariate analyses such as partial least squares, will be used to relate MRI findings with clinical, olfactory, behavioural, and electrophysiological outputs.

Results

To date, 44 participants (32 COVID-19 patients, 5 hospitalized; and 12 controls) have had initial assessment and approximately half of each group have had follow-up. Common symptoms across the whole sample were: fatigue, fever, cough, and sore throat. Preliminary MRI results are shown in Fig. 1.

Interpretation

The NeuroCOVID-19 observational study investigates neurological and lung effects associated with COVID-19 in patients who were either hospitalized or self-isolated while infectious, compared to a novel matched control group of COVID-19 negative individuals who experienced cold or flu-like symptoms. Longitudinal follow-up at 3 months is used to assess whether effects linger or resolve.

Knowledge Gap. There are numerous neuroimaging reports of hospitalized COVID-19 patients with vascular and inflammatory lesions. However, much less is known about whether neuroimaging, behavioral, and electrophysiological alterations occur among COVID-19 individuals who were not hospitalized and self-isolated while infectious. Triaging COVID-19 patients has been an essential part of pandemic plans, however "hospitalized versus self-isolated" may be a false dichotomy in terms of the brain. COVID-19 may impact the brain in the absence of respiratory symptoms (29), and cerebrovascular lesions and/or microstructural alterations can occur insidiously, both with and without overt behavioural symptoms. Hence there is a strong possibility that COVID-19-related brain burden is underestimated in the general population. NeuroCOVID-19 addresses this knowledge gap. The initial anatomical MRI data suggest that COVID-19 participants can show brain impact from their disease over a broad age range, including young and middle-aged adults. The effects are not limited to hospitalized individuals and include those that were self-isolating with limited healthcare while infectious.

Limitations. First, diagnostic certainty of COVID-19 status is limited by the sensitivity and specificity of the PCR tests. Blood biomarkers of immunity status and other factors would provide important complementary information, and can be added to the protocol in the future. Second, recruitment bias can not be ruled out. Third, additional control groups are of interest: controls that have not experienced flu-like illness, to more broadly assess the impact of the pandemic on brain measures (30); and ICU patients that have not contracted COVID-19 but have been on a ventilator for an extended period, to address whether brain pathology and dysfunctions are unique to severe COVID-19 illness (31).

Conclusion. A growing body of literature supports notions of neuroinvasion and/or deleterious brain changes resulting from COVID-19 in some individuals. The extent of these changes and their time courses remain to be determined across the general population - not just in hospitalized individuals. The NeuroCOVID-19 protocol is designed to address this issue and to foster scientific collaboration and/or adoption at other sites to increase the scope of the study findings, using innovative methodology. For

example, the MRI protocol includes anatomical spatial resolution that exceeds typical imaging, such that smaller-sized lesions can be detected. Lung MRI provides an innovative alternative to chest CT or x-ray while avoiding radiation dose. The extensive functional imaging is designed to increase sensitivity to altered brain physiology. Lastly, aspects of the sensory, behavioural and EEG tests are conducive to mobile or remote assessment.

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Summary of Tables and Figures, with text captions

Visit	Protocol / Assessment	Added description
1 (virtual, by phone)	Eligibility, consenting, COVID-19 test documentation	Review inclusion/exclusion criteria and MRI contraindication, collect demographics, medical symptoms & history, and screen for compliance and illness status (24 hours prior to study visit).
2 (in hospital)	Brain and lung MRI, smell, vision, NIH toolbox assessments, EEG	
3 (virtual, by internet)	Mnemonic similarity task, PROMIS assessments	Complete initial assessments using online meeting platforms.
4 (in hospital; 3 month follow-up)	repeat visit 1	Contrast agent not injected at follow-up.
5 (virtual, by internet; 3 month follow-up)	repeat visit 2	

Table 1. Summary of visit assessments for the NeuroCOVID-19 study. Abbreviations are: COVID-19= coronavirus disease; MRI=magnetic resonance imaging; NIH toolbox=National Institute of Health toolbox for neurocognitive testing. EEG= electroencephalography. PROMIS= patient-reported outcomes measurement information system.

MRI Sequence	Parameters	Scan Time (min:sec)
3D MPRAGE (**M**agnetization **P**repared **RA**pid **G**radient **E**cho)	TR/TE/TI=2500/4.7/1100 ms; θ=7°; FOV=256×256×192 mm;1 mm isotropic voxels	3:45
3D T2-weighted FLAIR (**FL**uid-**A**ttenuated **I**nversion **R**ecovery)	TR/TE/TI=5000/388/1800 ms; FOV=256×256×192 mm;1 mm isotropic voxels	5:57
3D T2-weighted SPACE (**S**ampling **P**erfection with **A**pplication-optimized **C**ontrasts using different flip angle **E**volution)	TR/TE_{eff}=3200/408 ms FOV=240×240×176 mm 0.9 mm isotropic voxels	3:42
2D SWI (**S**usceptibility-**W**eighted **I**maging)	TR/TE=28/20 ms; FOV=240×240×156 mm Acceleration factor=2; 23.1% oversampling 0.625×0.625×3.0 mm voxels	4:02
2D Triple-shell DTI (**D**iffusion **W**eighted **I**maging)	TR/TE=4300/62 ms; FOV=240×240 mm; 60 slices 2.5 mm isotropic voxels; b=700,1400,2100 s/mm^2 30 gradient directions; 4 b=0 values; 1 average	8:34
3D pCASL (**P**seudo-**C**ontinuous **A**rterial **S**pin **L**abelling)	label duration=1500 ms ; post-label delay=1800 ms 3D echo planar turbo gradient spin echo readout TR/TE_{eff}=4100/37 ms; FOV=240×240×120 mm 2.5 mm isotropic voxels; 10% oversampling Turbo factor=14; background suppression	4:27
2D BOLD (**B**lood **O**xygenation **L**evel-**D**ependent) **resting state functional MRI**	2D echo planar imaging; TR/TE=2130/30 ms; flip angle=70° FOV=224×224×140 mm; 3.5 mm isotropic voxels 250 time points	9:00
UTE-VIBE (**U**ltra-short **TE** with **V**olumetric **I**nterpolated **B**reath-hold **E**xamination)	TR/TE=2.5/0.05 ms; θ=5.5°; coronal prescription 600×600×260 mm; 2.1×2.1×2.5 mm voxels	0:14
Contrast Agent Administration		
DSC (**D**ynamic **S**usceptibility **C**ontrast) **MRI**	2D echo planar imaging; TR/TE=1250/30 ms θ=7°; FOV=220×220×80 mm 1.74×1.74×4.0 mm voxels; 140 time points	3:07
UTE-VIBE (**U**ltra-short **TE** with **V**olumetric **I**nterpolated **B**reath-hold **E**xamination)	TR/TE=2.5/0.05 ms; θ=5.5°; coronal prescription 600×600×260 mm; 2.1×2.1×2.5 mm voxels	0:14
3D MPRAGE (**M**agnetization **P**repared **RA**pid **G**radient **E**cho), 10 min post-injection.	TR/TE/TI=2500/4.7/1100 ms; θ=7°; FOV=256×256×192 mm;1 mm isotropic voxels	3:45
Total Session Time		**56:33**

Table 2. MRI sequences, key acquisition parameters, sequence timing and protocol timing for the NeuroCOVID-19 study, undertaken at Sunnybrook Research Institute using a Magnetom Prisma 3T MRI system (Siemens Healthineers, Erlangen, DEU). 3D=three dimensional; 2D=two dimensional multislice; TR=repetition time;TE=echo time; TEeff=effective echo time; TI=inversion time; θ=flip angle; FOV=field of view. All sequences are part of the Siemens MRI product, with the exception of the pCASL implementation (32) and the lung MRI implementation (33). Lung MRI is performed immediately prior to intravenous administration of the contrast agent and immediately following the DSC MRI. 3D T1-weighted MPRAGE MRI is repeated following DSC MRI, 10 minutes after administration of contrast agent. Brain and lung MRI are conducted with the standard head coil and standard 18-channel body array, respectively.

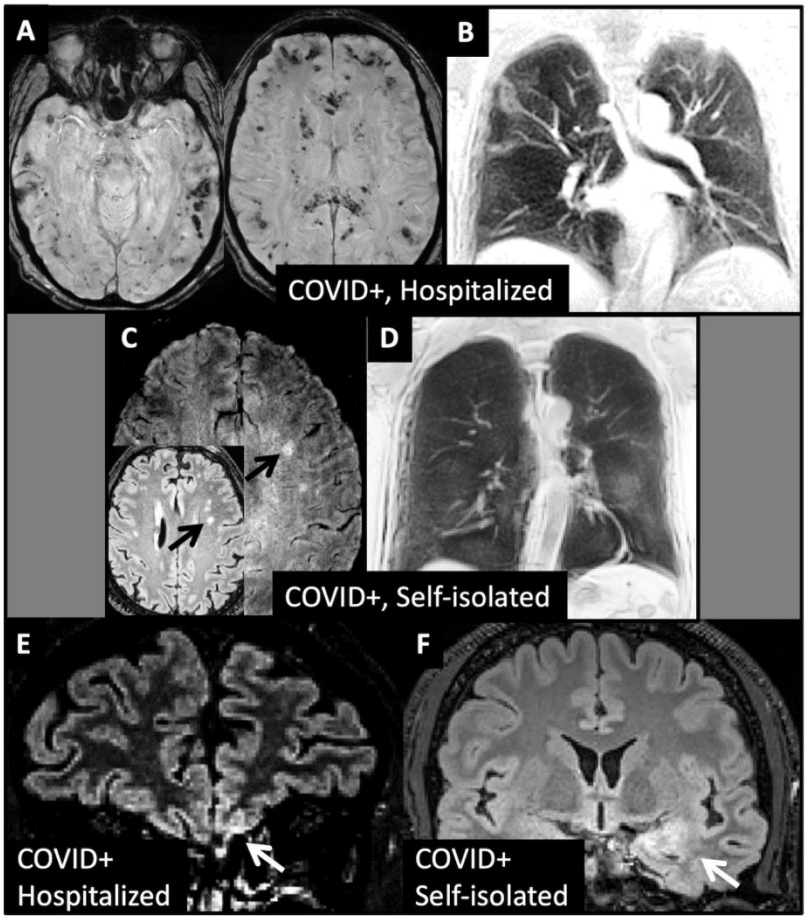

Figure 1. Brain and lung images from a selection of study participants. *Top*: a COVID+ male in his 50's who spent >12 days on a ventilator before recovering showed (**A**) numerous moderate sized lesions on SWI and (**B**) substantial abnormal signal intensities on lung imaging. This individual had severely impaired olfaction and moderate behavioural impairment (NIH toolbox 17th percentile on working memory, and complete inability to discriminate previously seen items from similar lures). He has been referred to a neurology clinic for follow-up. *Middle*: a COVID+ male in their 50's who self-isolated and showed micro-lesions in (**C**) SWI and T2 FLAIR imaging (inset), with (**D**) corresponding lung image with diffuse changes. Olfaction and sensory/behavioural tests were within age norms but self-report indicated difficulty in performing activities of daily living involving memory. *Bottom*: a COVID+ female in their 50's with moderately impaired olfaction and (**E**) elevated T2-FLAIR signal near the olfactory bulb and orbitofrontal cortex; and a COVID+ male in their 30's with an encephalitis-like lesion in the temporal lobe as observed (**F**) on T2-FLAIR imaging.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Herein, the checklist is completed using "GREEN" font with respect to:
CMAJOpen-2021-0023, entitled "NeuroCOVID-19: a longitudinal protocol for observing impact on brain structure and function in individuals recovering from COVID-19 after hospitalization or self-isolation
Submitted by MacIntosh et al.

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym **COMPLETED**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry **INTENDING TO REGISTER WITH clinicaltrials.org**
	2b	All items from the World Health Organization Trial Registration Data Set **N.A.**
Protocol version	3	Date and version identifier: **COMPLETED**
Funding	4	Sources and types of financial, material, and other support: **COMPLETED**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors: **COMPLETED**
	5b	Name and contact information for the trial sponsor: **N.A.**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities: **N.A.**
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee): **DATA MANAGEMENT TEAM is currently pending**

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention: **COMPLETED**
	6b	Explanation for choice of comparators: **COMPLETED**
Objectives	7	Specific objectives or hypotheses: **COMPLETED**
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory): **COMPLETED**

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained: **COMPLETED**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists): **COMPLETED**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered: **N.A. for this observational study**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease): **N.A.**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests): **N.A.**
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial: **N.A.**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended: **COMPLETED**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure): **COMPLETED**

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations: **COMPLETED**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size: **COMPLETED**

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions: **N.A. no randomization**
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned: **N.A.**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions: **N.A.**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how: **COMPLETED**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial: **COMPLETED**

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol: **COMPLETED**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols: **N.A.**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol: **COMPLETED**

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol: **COMPLETED**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) **COMPLETED**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation): **N.A.**
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed: **An ad-hoc data monitoring committee is in place however a formal committee is currently pending due to the nature of this observational study.**
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial: **N.A.**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct **N.A.**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor **N.A. this study does not involve a medicinal / therapy.**
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval: **COMPLETED**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) **COMPLETED**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) **COMPLETED**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable **COMPLETED**

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial **COMPLETED**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site **COMPLETED**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators **COMPLETED**
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation **N.A.**
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions **COMPLETED**
	31b	Authorship eligibility guidelines and any intended use of professional writers **N.A.**
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code **COMPLETED**
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates **COMPLETED**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable **COMPLETED**

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.