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Examining the Association between Brain MRI Measures at 7 Tesla and cognition following COVID-19 Infection

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Abstract

The long-term neuropsychological, cognitive, and neurobiological effects of the severe acute respiratory syndrome (SARS-CoV-2) in survivors with milder symptoms are still poorly understood. In this thesis we evaluated cognitive and psychological changes approximately five weeks after a wide range of symptoms in COVID-19 illness and determined whether advanced diffusion magnetic resonance imaging measures within subcortical brain structures of the limbic system were related to neurological, respiratory, psychiatric, and gastric symptoms experienced during the acute phase of illness. Cognitive and neuropsychological evaluations were performed in 45 participants who experienced neurological symptoms during the acute phase of COVID-19 illness. Participants also underwent a 7 Tesla MRI neurological exam on the same day. The group showed a significant reduction in attention compared to a normative population, but no differences in other cognitive domains. Although white matter hyperintensities were visible on fluid attenuated inversion recovery (FLAIR) images in 22 of 43 participants consistent with small vessel ischemic disease and migraine, this incidence is consistent with that expected in a normative population. Participants were divided into groups based on the presence or absence of symptoms at their acute illness from the medical history collected over the phone or in-person during recruitment. No differences were observed in subcortical brain structure volumes when comparing participants between subgroups. Differences in advanced diffusion metrics were observed within several subcortical structures ($p<0.0036$, Bonferroni corrected Mann-Whitney U-test) when comparing groups suggesting subtle tissue changes in several regions that were mostly related to respiratory and gastric symptoms. There were no strong associations between diffusion measurements and attention. Future studies should follow participants longitudinally to determine whether the observed changes persist.

Keywords

SARS-CoV-2, COVID-19, MRI, cognition, limbic system, diffusion, magnetic resonance imaging, diffusion tensor imaging, DTI

Summary for Lay Audience

We have struggled with coronavirus disease 2019 or COVID-19 since December 2019. This virus led to a pandemic because it has spread worldwide and affected many people. It impacted the world from a health and economic perspective and motivated scientists from all over the world to investigate the illness to minimize the risks to future human health.

Although COVID-19 starts by infecting the respiratory system, it causes a range of symptoms and affects many different organs, including brain. In this thesis, we investigate the long-term cognitive effects and imaging changes of COVID-19 infection in the brain within 45 patients with mostly mild symptoms after they have recovered from the respiratory symptoms. We used a very high magnetic field strength MRI scanner to acquire images of the brain for this study to better understand subtle changes in brain tissue.

To evaluate cognition, we compared COVID-19 survivors in the study using standardized questionnaires with data from a normative group that included healthy adolescents and adults. The results showed that patients who recovered from COVID-19 illness had attention deficits. In some patients, we observed minor changes in the MRI scans indicating some disease processes, but these were mostly consistent with what we would expect in a control group of people at the same age. Since we did not have access to baseline brain imaging, we cannot say that these abnormalities are directly related to the COVID-19 infection.

We also used more advanced diffusion MRI techniques to investigate microstructural changes in the brain. Here, we did find some changes within the brain tissue when comparing groups with and without specific symptoms that the patients experienced during their acute illness.

In summary, this study found changes in attention about five weeks (138±54 days) after COVID-19 illness and subtle differences in some measures of tissue microstructure in the brain. Larger studies are needed to confirm these findings and to determine if these changes persist.

Co-Authorship Statement

The following thesis contains material from posters that have been previously presented at several symposia and conferences and that will be submitted for publication in the near future. This project involved a large team of researchers from across London, without whom this study would not have been possible. The contributions of other co-authors are listed below. In my role as an MSc candidate, I was responsible for the acquisition of the majority of the presented data, administering and evaluating all in-person and over-the-phone cognitive assessments, scheduling and communicating with all study participants, implementing an imaging analysis pipeline to segment cortical structures in the brain, making diffusion measurements in subcortical brain structures, performing all data analyses, and preparing the relevant presentations and manuscript.

A portion of the material presented in Chapter 2 was presented at the Imaging Network of Ontario 2022 symposium and the International Society for Magnetic Resonance in Medicine 2021 annual meeting.

Dr. Megan Devlin was the clinical lead for this study, with additional clinical participation from Marko Mrkobrada, Michael Silverman, Erin Spicer, Stephen Pasternak, Luciano Sposato, Doug Fraser, Anthony Tang, and Elizabeth Finger. Stephanie Handsor was responsible for patient recruitment and clinical data management. Kristy Coleman and Lauryn Richardson provided support for study set-up. Dr. Ravi Menon provided oversight of the susceptibility weighted imaging acquisition. Dr. Corey Baron provided oversight of the diffusion tensor imaging protocol, advanced diffusion MRI and developed the analysis pipeline to create all diffusion tensor imaging metric images. All diffusion metric images were generated by Dr. Tales Santini. Dr. Michael Jurkiewicz performed all the clinical reads and interpretation of all imaging data. Dr. Elizabeth Finger oversaw all cognitive and neuropsychological testing and Koula Pantazopoulos helped to complete neurocognitive testing and scoring. Dr. Yves Bureau provided oversight and advice related to the statistical analyses performed within the study. Dr. Robert Bartha conceptualized the study and provided project oversight and guidance all stages of this work.

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I would like to thank those individuals who supported me unconditionally through the duration of my masters. The pandemic affected everyone's life and as a graduate student I could not have made it without the help and support of the people around me.

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My thanks to Dr. Corey Baron and Dr. Tales Santini for helping me understand the concepts of advanced diffusion MRI techniques in their lab.

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I would like to thank my advisory committee Dr. Jonathan Thiessen and specifically Dr. Elizabeth Finger who guided me in performing the cognitive assessments and concepts. I also want to thank Koula Pantazopoulos who was unconditionally thorough and helped me a lot from the beginning of this project with the RBANS and other cognitive assessments, and Stephanie Handsor, our research coordinator, for her tireless help with patient recruitment and managing patient information and medical histories.

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Table of Contents

Abstract ii

Summary for Lay Audience iii

Co-Authorship Statement iv

Acknowledgments v

Table of Contents vi

List of Tables ix

List of Figures x

List of Appendices xii

List of Symbols and Abbreviation xiii

Chapter 1 1

1 Introduction 1

1.1 Research Motivation and Objectives 1

1.2 Brain Subcortical Structures and the Limbic System 1

1.2.1 Hypothalamus 2

1.2.2 Hippocampus 2

1.2.3 Amygdala 2

1.2.4 The Basal Ganglia 3

1.2.5 The Caudate 3

1.2.6 The Putamen 3

1.2.7 The Globus Pallidus 4

1.2.8 The Nucleus Accumbens 4

1.3 Magnetic Resonance Imaging (MRI) 4

1.3.1 Ultra-high field (UHF) MRI 4

1.3.2 Magnetization prepared-rapid gradient echo (MPRAGE) 5

1.3.3 Fluid attenuation inversion recovery (FLAIR) 5

1.3.4 3D-Gradient echo (GRE) 6

1.3.5 3D-time-of- flight (TOF) angiography 6

1.3.6 Advanced diffusion MRI (dMRI) 6

1.3.7 Diffusion kurtosis imaging (DKI) 8

1.4 Cognitive Assessments 9

1.4.1 The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) 9

1.4.2 The Montreal Cognitive Assessment (MoCA) 10

1.4.3 The Telephone Interview for Cognitive Status (TICS) 11

1.4.4 Columbia Suicide Severity Rating Scale (CSSRS) 11

1.4.5 Diagnostic and Statistical Manual of mental disorders, Fifth Edition (DSM-5) 11

1.5 SARS-CoV-2 12

1.5.1 COVID-19 Infections in CNS 14

1.5.2 Neurological Manifestation in COVID-19 Patients 15

1.5.3 Neurological Side Effects Associated with COVID-19 Treatment 21

1.5.4 Neuropsychiatric Complications of COVID-19 Illness 22

1.5.5 Post-Acute COVID-19 Syndrome 24

1.5.6 Brain Microstructural Changes in COVID-19 Patients 24

1.6 Thesis Objectives 25

Chapter 2 27

2 Examining the Association between Brain MRI Measures at 7 Tesla and cognition following COVID-19 Infection 27

2.1 Introduction 27

2.2 Materials and Methods 30

2.2.1 Study Design 30

2.2.2 Participants 31
2.2.3 Neuropsychological Assessments 32
2.2.4 Magnetic Resonance Imaging 33
2.2.5 Diffusion MRI 34
2.2.6 Statistical analysis 36
2.3 Results 37
2.3.1 Demographic and Clinical Characteristics 37
2.3.2 MRI Clinical Interpretation 39
2.3.3 Cognitive and Neuropsychological Evaluation 42
2.3.4 MRI Diffusion Changes Associated with Cognitive Performance 45
2.3.5 MRI Diffusion Differences Associated with Neurological Symptoms 46
2.4 Discussion 49
2.5 Strengths and Limitations 53
2.6 Conclusions 53
Chapter 3 55
3 Objectives, Conclusion, and future direction 55
3.1 Objectives 55
3.2 Conclusion 56
3.3 Future direction 57
Appendices 79
Curriculum Vitae 81

List of Tables

Table 2-1 : 7T MRI Protocols and acquisition parameters 33

Table 2-2 : Clinical Symptom Frequency 38

Table 2-3 : MRI Findings and Frequency 40

Table 2-4 : Neuropsychological Characteristics 42

Table 2-5 : Correlations between RBANS Indices and Demographic Variables 43

Table 2-6 : Severity of Neuropsychological Symptoms 43

Table 2-7: Significant Neuropsychological Findings Related to Clinical Characteristics 45

Table 2-8 : Correlation between RBANS and Diffusion Metrics 46

Table 2-9 : Correlation Between Age and Subcortical Volume 46

Table 2-10 : Diffusion MRI findings related to clinical characteristics 47

Table 2-11: Partial correlation between Diffusion Metrics and Onset Days 49

List of Figures

Figure 1-1: The limbic system. Lateral view of the brain showing some of the limbic system structures. Image by Marieb et al. [173]. .....**Error! Bookmark not defined.**

Figure 1-2: Basal ganglia. Three-dimensional view of the basal ganglia, deep in the cerebrum. Image by Marieb et al. [173] .....**Error! Bookmark not defined.**

Figure 1-3: The five domains and the subtests that contribute to each domain [114]. 10

Figure 2-1: Study Procedures and Schedule of Events 31

Figure 2-2: T1-weighted anatomical MP2RAGE images showing the subcortical regions included in the current study: A: sagittal view, B: coronal view, C: axial view. 1: Brain stem; 2: right thalamus; 3: left thalamus; 4: right caudate; 5: left caudate; 6: right putamen; 7: left putamen; 8: right pallidum; 9: left pallidum; 10: left hippocampus; 11: right hippocampus; 12: right accumbens; 13: left amygdala. 35

Figure 2-3: Description of the subcortical segmentation pipeline highlighting μFA diffusion MRI analysis within the caudate. A: T_1-weighted axial image with subcortical structures shown with colors corresponding to Figure 2. B: Corresponding μFA map. C: The corresponding T_1-weighted anatomical image. D: Left caudate mask (white pixels) and left caudate mask after 2 voxels erosion to minimize partial volume effect (red pixels). E: the final left caudate mask used to calculate mean μFA within the left caudate, superimposed on the T_1-weighted anatomical image. 36

Figure 2-4: Examples of WM hyperintensity and abnormal susceptibility. A: Axial GRE (slice thickness 1.25mm) of a 35-39 year old man with a punctate focus abnormal susceptibility on the left superior temporal gyrus; B: Axial GRE (slice thickness 1.25mm) of a 35-39 year old woman with a developmental venous anomaly in the right anterior frontal lobe; C: Axial FLAIR image (slice thickness 0.8mm) of an 18-24 year old woman D: Axial FLAIR image (slice thickness 0.8mm) of a 35-39 year old woman; E: Axial FLAIR image (slice thickness 0.8mm) of a 30-34 year old woman. All three FLAIR images show nonspecific foci of hyperintensities in the bilateral cerebral WM with frontal lobe

predominance commonly seen in patients with migraines. Age ranges are provided to maintain anonymity. 41

Figure 2-5: Significant diffusion MRI findings in subcortical structures related to symptoms present at the time of acute illness 48

List of Appendices

Appendix A 1: Ethics Approval for Human NeuroCOVID study

List of Symbols and Abbreviation

MD	Mean Diffusivity
RD	Radial Diffusivity
AD	Axial Diffusivity
K_{lin}	Linear Kurtosis
ANOVA	Analysis of Variance
DTI	Diffusion Tensor Imaging
DWI	Diffusion Weighted Imaging
FA	Fractional Anisotropy
μFA	Microscopic Fractional Anisotropy
FSL	FMRIB Software Library
T_1	Longitudinal Relaxation
T_2	Transverse Relaxation
WM	White Matter
GM	Grey Matter
RBANS	Repeatable Battery for the Assessment of neuropsychological Status
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
TICS	Telephone Interview for Cognitive Status
MoCA-Blind	Telephone-Based Montreal Cognitive Assessment
APA	American Psychiatric Association Assessment Measures
CSSRS	Columbia Suicide Severity Rating Scale
PROMIS	Patient-Reported Outcomes Measurement Information System
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
COVID-19	Coronavirus disease 2019
PTSD	Post-Traumatic Stress Disorder

Chapter 1

1 Introduction

1.1 Research Motivation and Objectives

Due to the novelty of the coronavirus disease of 2019 (COVID-19), the long-term consequences of the infection on respiratory function and its impact on other organs including the brain requires further investigation. The aim of the work presented in this thesis was to understand the long-term cognitive and neurobiological effects of COVID-19 in patients with mild infection who experienced neurological symptoms, after the respiratory symptoms had resolved.

1.2 Brain Subcortical Structures and the Limbic System

The complex and diverse range of neurological symptoms encountered by patients following COVID-19 infection suggests a broad potential impact of the virus in the brain. Although the brain may be globally affected by SARS-CoV-2 infection and the subsequent cytokine storm, the limbic system may be particularly vulnerable to damage.

The limbic system is a set of structures on both sides of the thalamus under the cerebrum. It includes the hypothalamus, the hippocampus, the amygdala, and several other nearby areas. The limbic system is primarily responsible for emotion and the formation of memories.

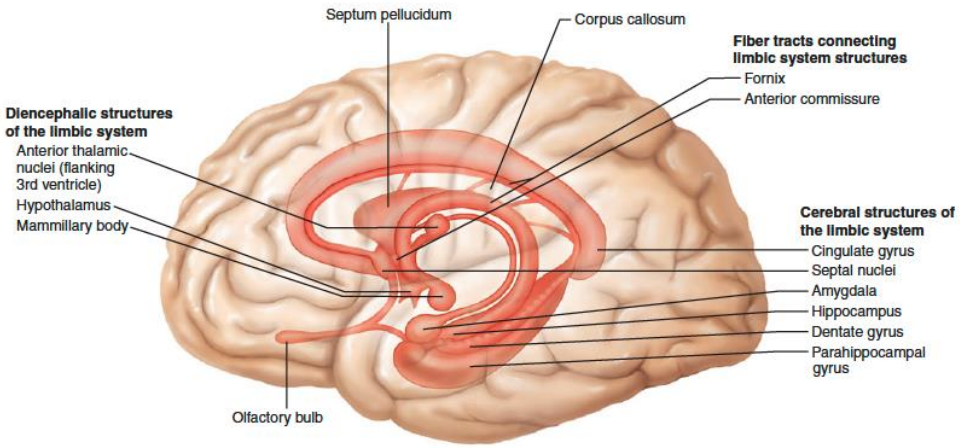


Figure 1-1: The limbic system**.** Lateral view of the brain showing some of the limbic system structures. Image by Marieb et al. [173].

1.2.1 Hypothalamus

The hypothalamus is located below the thalamus on both sides of the third ventricle. The hypothalamus is mainly responsible for homeostasis which is the process of returning something to some set point. It is responsible for regulating hunger, thirst, response to pain, levels of pleasure, anger, and aggressive behavior. It also regulates the functioning of the autonomic nervous system that controls blood pressure, breathing and response to emotional circumstances. The hypothalamus receives inputs from several sources. For example, it gets information from the limbic system and the olfactory nerves that helps regulate eating.

1.2.2 Hippocampus

The hippocampus resembles a horn that curves back from the amygdala. It is an important structure involved in the conversion of short-term memories into long-term memories. If the hippocampus is damaged the formation of new memories will be impaired. Hippocampal damage is for example, an important component in the progression of Alzheimer's disease.

1.2.3 Amygdala

The amygdala is an almond-shaped structure full of neurons found on either side of the thalamus at the lower end of the hippocampus. It is important in experiences of anger and fear. The amygdala impacts on the formation of memories of experiences based on emotional impact.

Beyond the hypothalamus, hippocampus, and amygdala there are other areas near the limbic system that are connected to it including the cingulate gyrus, ventral tegmental area, the basal ganglia, and the prefrontal cortex.

1.2.4 The Basal Ganglia

The basal ganglia include the caudate nucleus, the putamen, the globus pallidus and the nucleus accumbens. The basal ganglia are adjacent to the thalamus and connected with the cortex. All these structures exist bilaterally, one set on each side of the central septum. They are involved in repetitive behaviors, reward experience and focusing attention.

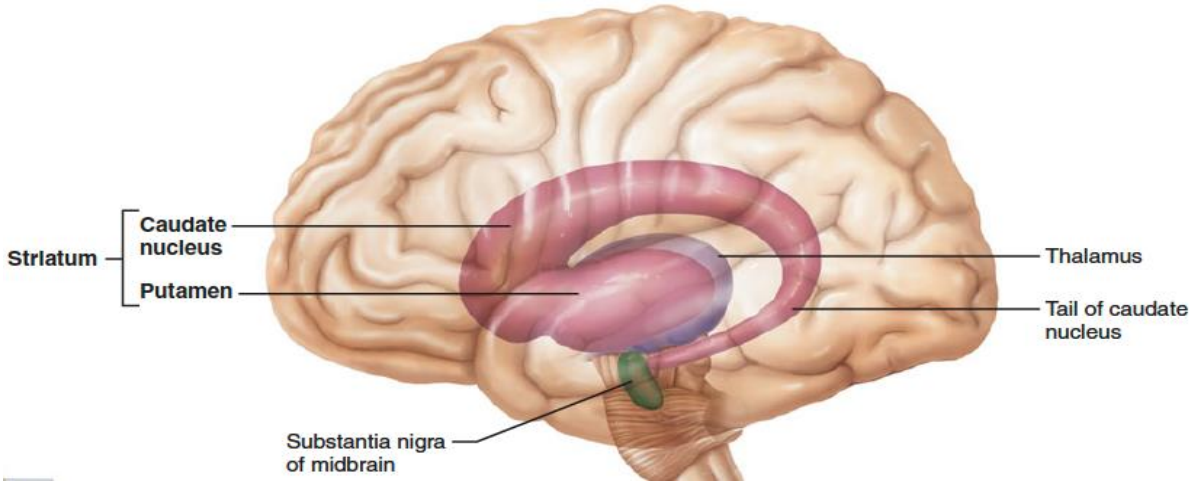


Figure 1-2: Basal ganglia. Three-dimensional view of the basal ganglia, deep in the cerebrum. Image by Marieb et al. [173]

1.2.5 The Caudate

The caudate nucleus is a C-shaped structure in the center of the brain. It is divided into three parts: the head, body, and tail. It plays a role in repetitive function, and it is involved in mental disorders including attention deficit and hyperactivity disorder (ADHD) [87].

1.2.6 The Putamen

The putamen is a subcortical structure that forms the dorsal area of the basal ganglia. It is responsible for reinforcement learning and motor control including speech articulation [88].

1.2.7 The Globus Pallidus

The globus pallidus (GP) or pallidum is a triangular mass of cells medial to the putamen. The main function of the GP is to control conscious and proprioceptive movements. It receives information from multiple structures [89]. The involvement of the GP has demonstrated in several different disorders such as obsessive-compulsive disorders (OCD), ADHD, Parkinson's, and Huntington's [89]– [91].

1.2.8 The Nucleus Accumbens

The nucleus accumbens is the most inferior part of the striatum that is connected mainly to the limbic system. Together with the prefrontal cortex and amygdala it consists of a part of the cerebral circuit that regulates functions associated with effort. It provides emotional and behavioral components of feelings. It interferes between motivation and action and plays a key role in food intake, sexual behavior, reward-motivated behavior, stress related behavior, and substance-dependence. It also involved in several cognitive and emotional functions, and some severe psychiatric disorders such as depression, schizophrenia, addiction, attention deficit disorders and other anxiety disorders, and post-traumatic stress disorder (PTSD) [92].

1.3 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging is based on the nuclear magnetic resonance (NMR) phenomenon, which is dependent on the property of nuclear spin. NMR involves measuring emitted energy from atomic nuclei placed in a magnetic field following excitation by radio frequency (RF) waves. Hydrogen is the most abundant element in the human body that can generate an MRI signal [95].

1.3.1 Ultra-high field (UHF) MRI

From the initial grainy images of the human brain, technical developments in MR imaging techniques and improved hardware now produce unique and very detailed images of brain anatomy, function and metabolism that are an integral component of neurologic evaluation [96]. The growing interest in ultra-high field MRI is related to the potential to improve clinical results with better quality images due to increased signal-to-

noise ratio (SNR). SNR increases with the magnetic field strength of the MRI scanner with potential for higher spatial resolution and contrast compared to lower field strengths (1.5T or 3T) to improve lesion detection [97]. A downside of the increase in field strength is the introduction of non-uniformities in the transmit field radiofrequency that can compromise image contrast uniformity [98]. Ultra-high field MRI applications in neuroimaging include detection of changes in cortical structures like microinfarcts and cortical plaques in multiple sclerosis, imaging of the hippocampus with high spatial resolution, iron accumulation, and vascular imaging [97]. In the study presented in this thesis, five major types of imaging contrasts were obtained in participants as described below.

1.3.2 Magnetization prepared-rapid gradient echo (MPRAGE)

MPRAGE consists of a non-selective (180°) inversion pulse followed by a series of rapid acquisition of gradient echoes obtained at short echo-times (TE) and small flip angles that makes it the most common sequence for 3D-T1-weighted imaging. MP2RAGE by comparison includes two gradient readouts between inversion pulses.

The MPRAGE sequence is one of the most common T1-weighted (T_1-w) image acquisition for structural brain imaging that provides high contrast between grey and white matter specially for brain segmentation. The self-bias-field corrected MP2RAGE is used at 7T to improve the signal inhomogeneity [98] by combining the data from both readouts.

1.3.3 Fluid attenuation inversion recovery (FLAIR)

FLAIR is an MRI sequence preceded by an inversion pulse that incorporates an inversion recovery (IR) period to null fluids. In brain imaging, better detection of periventricular WM hyperintensities is possible when cerebrospinal fluid (CSF) is nulled within an image [99]. For optimal suppression, an inversion time is selected that corresponds to the time needed for the CSF magnetization to reach a null point after inversion. At that point there is no longitudinal magnetization from CSF to contribute to subsequent imaging. When an excitation pulse is applied, since there is no longitudinal component from the

CSF, no transverse magnetization is generated after excitation and the CSF signal is minimized [99].

1.3.4 3D-Gradient echo (GRE)

Gradient echo (GRE) is a fast MRI technique. The small flip angle that is employed in GRE, allows for the use of a short repetition time (TR), which can decrease scan time. Thus, GRE can be used for rapid volumetric imaging of thin continuous slices (three-dimensional imaging) without cross-talk [100].

The use of gradient echoes (rather than 180° refocusing pulses) in GRE imaging results in greater dephasing of spins and makes this pulse sequence sensitive to magnetic field distortions. As a result, GRE is very effective in identifying microhemorrhages [100].

1.3.5 3D-time-of- flight (TOF) angiography

Time-of- flight (TOF) MR angiography (MRA) is a common non-invasive method used to visualize the human vascular system. Contrast is based on flow-related enhancement using 2D or 3D gradient echo techniques [100]. 3D-TOF MRA has higher SNR compared to 2D since the signal is acquired from a larger volume. It also is capable of a higher spatial resolution [100]. TOF-MRA at high field strength (e.g. 7T) is advantageous over lower field MRI because T_1 relaxation time constants increase at high field and a better vessel to background contrast can be achieved [101]. Furthermore, as mentioned before, UHF scanners provides higher SNR and spatial resolution [97] that enhance the sensitivity of the TOF angiogram and consequently the detection of vascular abnormalities [102].

1.3.6 Advanced diffusion MRI (dMRI)

Diffusion refers to the movement of water molecules in the extracellular space due to random thermal motion. The motion is restricted either by cellular boundaries such as ligaments and membranes or pathology. The net displacement of molecules diffusing across an area of tissue per second is the apparent diffusion coefficient (ADC). If diffusion is restricted, ADC is low, while in areas of free diffusion it is high.

In diffusion weighted imaging (DWI), the sequence is sensitized to this motion by applying two gradients on either side of a 180° RF pulse. In diffusion imaging, normal tissue with high ADC has lower signal intensity compared to abnormal tissue, which may have low ADC. The diffusion of water is often restricted in pathology.

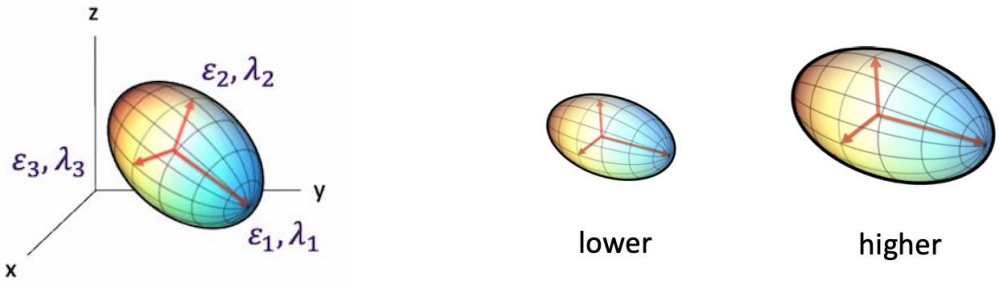
1.3.6.1 Diffusion tensor imaging (DTI)

DTI is a quantitative MRI method to measure water movement within the tissue microstructure [103]. DTI is an extended form of DWI, that measures water diffusion in three gradient directions for an estimation of the trace of the diffusion tensor [104]. It is the most established technique for the non-invasive investigation of the CNS microstructure [105]. Microstructural features that affect the diffusion rate of water include cell size, shape, density, orientation and the presence of membranes and barriers [106], [107].

In DTI the signal is dependent on the magnitude and direction of water diffusion. Each voxel is fit with an ellipsoid that is represented by three unit vectors (ε_i =direction) and their corresponding length (λ_i =magnitude). There are two commonly derived quantitative measures from the tensor that inform us about cellular microstructure: fractional anisotropy (FA) and mean diffusivity (MD). To obtain these measures, the diffusion MRI data is fitted to the diffusion tensor model [108]. We use the ellipsoid components to describe water diffusion.

1.3.6.1.1 Mean diffusivity (MD)

MD is a measure of the average molecular motion independent of any tissue directionality and it is affected by cellular size and integrity [109]. The MD is defined in the equation provided below.

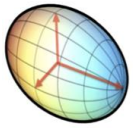


Mean Diffusivity (MD) = $(\lambda_1+\lambda_2+\lambda_3)/3$

1.3.6.1.2 Fractional anisotropy (FA)

FA is a measure of anisotropic water diffusion and reflects the degree of directionality of cellular structures (e.g. fiber tracts) and their structural integrity [104]. This value is obtained from the magnitude of the diffusion tensor due to anisotropy [110]. In a purely isotropic media, FA would be 0 and with increasing anisotropy the value tends to 1 [104].

$$FA = \sqrt{\frac{1}{2}}\frac{\sqrt{(\lambda_1-\lambda_2)^2+(\lambda_2-\lambda_3)^2+(\lambda_1-\lambda_3)^2}}{\sqrt{\lambda_1^2+\lambda_2^2+\lambda_3^2}}$$



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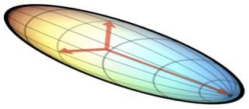

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1.3.6.1.3 Microscopic fractional anisotropy (μFA)

In some situations, conventional DTI is unable to distinguish between true microstructural complexity and neuron fiber orientation dispersion. This limitation can reduce specificity for disease in brain regions that have crossing or fanning axons [111]. Microscopic anisotropy (μA) is an anisotropy metric that is independent of reference frame and orientation dispersion. Microscopic fractional anisotropy (μFA) is a normalized variation of μA that removes the dependence on compartment size [112].

1.3.7 Diffusion kurtosis imaging (DKI)

Diffusion kurtosis imaging is a method to quantify water diffusion in biologic tissue that is non-Gaussian. It is an extension of conventional diffusion-weighted imaging that requires a modified postprocessing method and higher *b* values[113]. The reason that DKI provides specific measures of tissue structure is because tissue structure is responsible for the deviation of water diffusion from Gaussian behavior, which is typically seen in homogenous solutions. This approach provides an estimate of both the Gaussian distribution (diffusion tensor metrics) and the deviation from this Gaussian

distribution (diffusion kurtosis metrics), which makes the method potentially more sensitive to visualize microstructural changes [113].

1.4 Cognitive Assessments

Standardized cognitive assessments provide a means to compare cognitive performance in people over time, or in people with neurological conditions or diseases. Although useful for assessing general changes, cognitive assessments can be variable and impacted by factors such as sleep, nutrition, fatigue, and the conditions of test administration. A series of standardized tests were used in this thesis to measure cognitive performance and mental state as described below.

1.4.1 The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) is a brief test that measures attention, language, visuospatial/constructional abilities, and immediate and delayed memory. The test has 12 subtests and takes about 20 to 30 minutes to complete, which maximizes patient cooperation and minimizes the effects of fatigue on patient performance. Although initially the RBANS was primary used for the assessment of dementia in older populations, its potential for screening neurocognitive status in younger patients became apparent and standardized data was modified to include normative reference data from ages 12 to 89 [114]. The normative scores were developed using a stratified, national sample of 690 healthy adolescents and adults. The RBANS can measure discrete neuropsychological domains by producing scaled scores. Furthermore, alternate forms make it possible to evaluate disease progress or outcomes following treatment with therapeutics or rehabilitation [114].

1.4.1.1 Organization of the Scale

The RBANS tests five different domains (Figure 3). The score for each of the 12 subtest contributes to one of these five domains. A total score can be computed by combining the five domain scores.

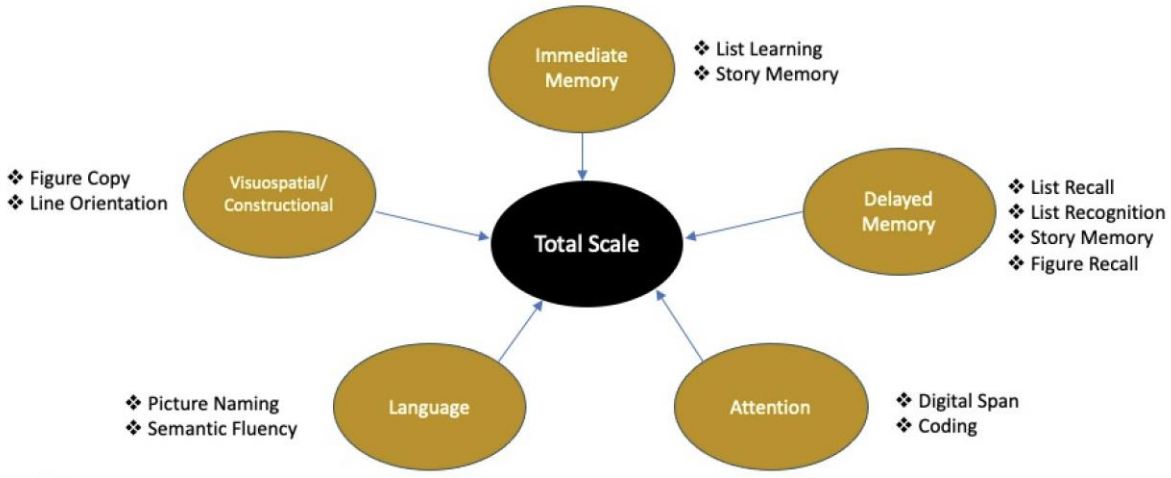

Figure 1-3: The five domains and the subtests that contribute to each domain [114].

1.4.2 The Montreal Cognitive Assessment (MoCA)

The Montreal Cognitive Assessment (MoCA) is a rapid screening instrument for mild cognitive dysfunction. Mild cognitive impairment (MCI) is an intermediate clinical state between normal cognitive aging and dementia, which in many cases precedes dementia [115]. This tool assesses different cognitive domains including attention and concentration, executive function, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation.

It is a one-page test (available at www.mocatest.org) administered in 10 minutes with a maximum total score of 30 points. A score of 26 or above is considered normal. The MoCA-BLIND test is an adapted version of the original MoCA test that contains the same items except for those that require visual abilities, so it can be administered over the phone. This test takes approximately 5-10 minutes to complete with a total score of 22. A score of 19 or above is considered normal. (www.mocatest.org)

1.4.3 The Telephone Interview for Cognitive Status (TICS)

The Telephone Interview for Cognitive Status (TICS) is a global mental status test that can be administered over the phone or in-person [116]. It measures orientation, concentration, short-term memory, language, praxis, and mathematical skills. The test has excellent sensitivity and specificity in differentiating patients with Alzheimer's disease from normal and high test-retest reliability in this population [116].

1.4.4 Columbia Suicide Severity Rating Scale (CSSRS)

Suicide is one of the most important public health issues in many countries. Suicide risk increases in people with mental disorders or impulsive behavior, and those facing stressful situations [117]. The Columbia Suicide Severity Rating Scale (C-SSRS) has been widely used for assessment of suicidality by several agencies such as Health Canada and is available for free at www.cssrs.columbia.edu [117]. This scale assesses the worst point and lifetime severity and intensity of suicidal ideation and the type and lethality of suicidal behavior [117]. Selected items in the survey predict social risk including preparatory activity [118].

1.4.5 Diagnostic and Statistical Manual of mental disorders, Fifth Edition (DSM-5)

The Diagnostic and Statistical Manual of mental disorders, fifth edition (DSM-5) is the 2013 update to the Diagnostic and Statistical Manual of mental disorders published by the American Psychiatric Association. It provides guidelines for clinical evaluations at the time of initial patient visit and for monitoring treatment progress. The Patient-Reported Outcomes Measurement Information System (PROMIS) is a collection of person-centered measures of physical, mental, and social health [119] used to capture symptoms associated with mental disorders. The following measures were included in this thesis: DSM-5 Self-Rated LEVEL 1 Cross-Cutting Symptom Measure-Adult, LEVEL 2 Somatic Symptom-Adult Patient (adapted from the Patient Health Questionnaire Physical Symptoms [PHQ-15]), LEVEL 2 Sleep Disturbance-Adult (PROMIS-Sleep Disturbance-Short Form), LEVEL 2 Depression-Adult (PROMIS Emotional Distress-Depression-Short Form), LEVEL 2 Anxiety-Adult (PROMIS

Emotional Distress-Anxiety-Short Form), LEVEL 2 Anger-Adult (PROMIS Emotional Distress-Anger-Short Form), Severity of Post-traumatic Stress Symptoms-Adult (National Stressful Events Survey PTSD Short Scale [NSESSS]), and the Severity of Acute Stress Symptoms-Adult (National Stressful Events Survey Acute Stress Disorder Short Scale [NSESSS]).

1.5 SARS-CoV-2

Coronaviruses are a diverse group of viruses that infect animals and humans. In 2002 and 2012 two different coronaviruses caused fatal respiratory illnesses in humans and made coronaviruses a major public health concern in the twenty-first century [1] . In late December 2019 a novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) emerged in the city of Wuhan, China and spread quickly causing a global pandemic. SARS-CoV-2 is a highly transmissible coronavirus that can cause acute respiratory disease in humans [2] with a wide range of symptoms including fever, fatigue, cough and chest discomfort with dyspnea and bilateral lung infiltration in severe cases [3]. Additional symptoms may include sputum production, pneumonia, headache, hemoptysis, diarrhea, anorexia, sore throat, chest pain, chills, nausea, and vomiting [4]. The incubation period for the illness, typically called COVID-19, is 1-14 days, with dyspnea and pneumonia typically developing around 8 days from illness onset [5]. Olfactory and taste disorders have also been reported by some patients [6]. Approximately 40-50% of people infected with COVID-19 may be asymptomatic [7]. Neurological symptoms associated with COVID-19 infection are also frequently reported [8]– [11]. But it remains unknown whether the infection causes long-lasting alterations or damage in the brain. Such an effect could have a serious impact on the vulnerability of the brain and trajectory of other neurological diseases. Furthermore, psychiatric symptoms observed in COVID-19 patients that persist for a long time after recovery necessitate periodic monitoring of psychiatric symptoms and psychosocial support and treatments for survivors from the acute to chronic stages [10]. In this thesis, we begin to assess the microscopic tissue integrity of the brain in-vivo using advanced diffusion tensor imaging (DTI) methods with 2mm isotropic voxel, following COVID-19 infection

with ultra-high-field 7 Tesla MRI scanner and the association of these changes in the brain with long-term cognitive and neuropsychological dysfunction.

The way in which people experience COVID-19 infection varies significantly from individual to individual. Based on the cohort study by Cummings and colleagues, 67% of critically ill patients were men [5]. Men over 60 years of age with co-morbidities were at higher risk of developing severe respiratory symptoms that needed hospitalization or that led to death while most young people and children had milder symptoms or were asymptomatic [5]. Hypertension and diabetes mellitus were the most common co-morbidities among hospitalized COVID-19 patients [12]. The experience and severity of symptoms also varies in association with different SARS-CoV-2 variants.

In patients admitted to hospital bilateral multi lobar ground-glass opacity in the lungs was the most common radiologic features in their chest computed tomography (CT) [4]. Although SARS-CoV mostly affects the upper respiratory tract, SARS-CoV-2 can also target cells in the lower airway [13]. Ground-glass opacity (GGO) describes areas of hazy increased lung opacity through which the vessels and bronchial structures can still be seen. In most patients, marked lymphopenia has been observed, which is similar to what has been observed with other coronaviruses and it is particularly severe in non-survivors. COVID-19 infection leads to a high production of cytokines by white blood cells compared to other health conditions [14]. Patients admitted to ICU may have very high levels of cytokines caused by a cytokine storm [13]. In men over the age of 68 there is a higher risk of respiratory failure, acute cardiac injury and heart failure that leads to death, regardless of any history of cardiovascular disease [15]. As of the writing of this thesis, there have been 6 waves [16] associated with the COVID-19 pandemic in Ontario, Canada. Several different variants of the SARS-CoV-2 virus have been encountered, each with slightly different symptom profiles and severity.

Although the virus most commonly affects the respiratory system, it can affect any organ in the human body. The virus is an RNA virus, consequently replication can take several days to reach viral loads high enough to damage organs [17]. The virus binds to angiotensin converting enzyme 2 (ACE2) receptors in tissue membranes of most organs

including vascular endothelial cells, lung, heart, brain, kidneys, intestine, liver, and pharynx and can directly injure theses organs or cause systemic disorders or organ malfunction [13]. The ACE2 receptor is present in mucosa as well allowing the virus to enter the body through the eyes, nose, and mouth [18]. Coagulation disturbances and damage to vascular endothelium can contribute to multiple organ injury long after acute infection and lead to chronic injury. SARS-CoV-2 damages endothelial cells in organs and causes diffuse lymphocytic endothelitis which can lead to vasoconstriction [19]. The cytokine storm induced by the virus can also lead to a systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome (ARDS), multiple organ injury, shock, and death [14]. Beyond inflammation, hypercoagulability, and edema can also cause hypoperfusion and lead to organ ischemia [20]. Although primarily considered a respiratory virus, infiltration of the SARS-CoV-2 virus into the brain can produce a number of neurological symptoms.

1.5.1 COVID-19 Infections in CNS

Many viruses can enter the human central nervous system (CNS) [21]. Among these, coronaviruses not only target the respiratory tract but may also invade the CNS and cause neurological disease, which has been documented for almost all coronaviruses including severe acute respiratory syndrome coronavirus (SARS-CoV) [22]. It is still unknown whether the potential neuro invasion of SARS-CoV-2 has a direct effect on the acute respiratory failure of some COVID-19 patients [23].

Many patients with severe respiratory symptoms also have neurological symptoms that many be due to viral RNA-induced neural inflammation, or stroke triggered by coagulation, or impaired brain clearance. Although the most common symptoms of SARS-CoV-2 infection are respiratory in nature, some patients develop neurological symptoms including headache, nausea, vomiting, and loss of taste or smell. Neurological manifestations can also include impaired consciousness and delirium providing further evidence that the virus can spread to the human neocortex in the brain [24]. In fact, the presence of viral-like particles in the frontal tissue and capillary endothelium has been confirmed by electron microscopy [24]. SARS-CoV has been reported to infect the brain in both human and animal experimental studies [23]. Moreover, some coronaviruses

spread through a synapse-connected pathway to the medullary cardiorespiratory center from receptors in the lung and lower respiratory airways [23].

It has been suggested that a hematogenous route is the most probable pathway for the virus to enter the brain [9], [24]. The lymphatic drainage system in the brain that contains olfactory/cervical lymphatic vessels could also be an entry of SARS-CoV-2 to the brain [9]. ACE2 is present in the cerebral cortex and brain stem neurons and plays a role in the regulation of physiological functions including cardiovascular, metabolic, neurogenesis, and stress response [25]. Since ACE2 is expressed in the olfactory epithelium, the virus may enter the brain through axons of the olfactory bulb neurons under the cribriform plate [23]. The taste and smell disturbances that have been reported by some COVID-19 patients suggests olfactory bulb involvement in the illness. Based on the similarity between SARS-CoV-2 and previous coronaviruses responsible for respiratory syndromes including the Middle East respiratory syndrome (2012) and the severe acute respiratory syndrome epidemic (2003), it has been suggested that COVID-19 could be neuroinvasive [23]. In addition, there is evidence of edema and degeneration of neurons in the brain autopsy of patients with SARS [26]. Also, meningitis and encephalitis have been reported in some patients with COVID-19 infection, illustrating viral invasion of the CNS [14].

1.5.2 Neurological Manifestation in COVID-19 Patients

Since the beginning of the pandemic there has been concern that some COVID-19 survivors may be at a higher risk of neurological complications based on previous studies of other coronaviruses [27]. Neurological complications have been reported in previous respiratory syndrome pandemics [28], [29] during the acute phase of illness directly through viral infection or indirectly from the accompanying cytokine storm, or due to a post-infectious immune system response [30].

In addition to respiratory symptoms, neurological manifestations have been reported in COVID-19 patients including CNS, peripheral nervous system (PNS), and skeletal muscular injury manifestation [30]. CNS symptoms include dizziness, headache, impaired consciousness, acute cerebrovascular disease, ataxia, and seizure. PNS symptoms include taste and smell impairment, vision impairment and nerve pain. It is

worth mentioning that CNS symptoms were more common than PNS symptoms in COVID-19 patients [31]. Some of these neurological symptoms are non-specific such as headache, myalgia, and altered mental status while some are more specific syndromes that need urgent care [31]. These neurological symptoms may be explained by the presence of ACE2 in the nervous system and skeletal muscle [18]. Brain autopsies of patients with COVID-19 showed hyperemic and edematous brain tissue and some neuronal degeneration [32].

Most neurological manifestations occur in the early stages of the illness [31] and it has been shown that ischemic stroke can happen approximately two weeks after the onset of the illness [33]. Based on a study performed in the United States, the loss of taste or smell as a common symptom in COVID-19 infection, is more likely from the infection rather than other sequences of the infection [34].

Impaired consciousness varies from change of consciousness level such as somnolence, stupor, and coma to consciousness content such as confusion and delirium. Early diagnosis could prevent cross-infection, neurological injuries, and death especially in some patients that don't have typical symptoms such as fever, cough and diarrhea and come to the hospital with only neurological symptoms. Acute cerebrovascular disease including ischemic stroke and cerebral hemorrhage can be diagnosed by clinical symptoms and head CT. Diagnosis of seizure is based on clinical symptoms at the time of presentation. Skeletal muscle injury is when a patient experiences skeletal muscle pain and elevated serum creatine kinase levels [31].

Laboratory findings in patients with CNS symptoms have shown lower lymphocyte and platelet counts and higher blood urea nitrogen levels compared to patients without CNS symptoms which suggests immune suppression in patients with CNS symptoms, especially those with severe infection. In patients with less severe illness there were no significant differences in laboratory results between the patients with and without CNS symptoms [31]. Comparing lab findings of the patients with and without peripheral nervous system (PNS) symptoms in both severe and non-severe COVID-19 patients, did not show any significant difference [31].

Patients with skeletal muscle damage had higher level of creatine kinase regardless of the severity if their illness. They also had higher neutrophil counts and lower lymphocyte counts, which are representative of increased inflammatory and blood coagulation responses. The elevated proinflammatory cytokines in serum that cause some abnormalities in the nervous system may also be the source of some skeletal muscle damage [32].

D-dimer protein levels may represent how severe an infection is and might help to identify patients that are high risk of pulmonary complications and venous thromboembolism [35]. The D-dimer protein is a fibrin degradation product in the blood produced when a blood clot is degraded by fibrinolysis. The D-dimer concentration can help to diagnose pulmonary embolism and disseminated intravascular coagulation (DIC) in clinical practice [31]. A previous study of COVID-19 patients found the D-dimer levels were high in patients with muscle damage and severe infections, which may be the reason why patients with severe infection are more susceptible to cerebrovascular disease. Patients with muscle damage also had multiple organ damage including liver and kidney abnormalities [31].

Several studies have proposed that coronaviruses may also relate to CNS diseases such as multiple sclerosis and acute disseminated encephalomyelitis [36]. Multiple sclerosis (MS) is defined by patches of demyelination and inflammatory cell infiltration [37]. Coronavirus-like particles were found at autopsy in the brain tissue of MS patients [38] as well as human coronavirus RNA [39]. Also, in murine models, coronaviruses cause a chronic demyelination condition which looks like MS [40].

Acute disseminated encephalomyelitis (ADEMS) is a demyelination disease that invades the CNS and affects primarily children and young adults. It is detected on T1-weighted MR images as white matter hyperintensities in the brain and spinal cord. The symptoms in children include diffuse encephalopathy, seizures, optic neuritis, hemiparesis, and other symptoms supporting spinal cord transection. The disorder generally occurs as a para- or postinfectious process. Although there has been a report of a possible relation between COVID-19 infection and ADEM, there is no clear evidence of the relationship

between the infection agent and demyelination onset [40]. An experimental study in mice did show a relationship between coronaviruses and CNS demyelination [41] but supporting evidence of the relationship between the virus and demyelination in humans [40] is limited. Ann Yeh E *et al.* reported a case of a demyelination disease in a child with a positive PCR result for human coronavirus (HCoV) in cerebral spinal fluid and nasopharyngeal specimens.

Brain imaging findings in hospitalized COVID-19 patients include ischemic infarct, hemorrhages, and multiple patterns of leukoencephalopathy [31], [33]. There is also evidence that patients with acute lung injury are more prone to develop brain injury through hypoxemia and/or proinflammatory mediators between both the brain and the lung [42], [43].

1.5.2.1 Specific Neurological Complications

Beside non-specific neurological symptoms such as headache, fatigue and altered mental status in COVID-19 patients, more specific neurological symptoms have been also reported. Al-Ramadan and colleagues completed a literature review on the acute and post-acute neurological complications of COVID-19 illness which is summarized below [44].

1.5.2.1.1 Cerebrovascular Disease

The human cerebrovascular system includes arteries and veins that circulates blood flow to and from the brain. The carotid arteries and jugular veins are the main blood vessels in the brain and any occlusion or rupture in these vessels interferes with blood perfusion of the brain which could cause stroke with neurological deficits [45]. Stroke has been reported in several studies of COVID-19 patients. Some of the patients had hemiplegia with no medical history of comorbidities [46]. In a study by Beyrouti *et al.*, characteristic features of ischemic stroke have been described that showed large vessel occlusion in all patients and hypercoagulation in most patients. It is interesting that one of the patients had a stroke at the initial phase of the illness [47]. Ischemic stroke and hemorrhagic stroke were reported in another case series in Italy in which patients with severe illness developed stroke and some of them died or suffered from severe neurological disabilities

[48]. Notably, in an assessment of the severity of acute ischemic stroke in the Global COVID-19 Stroke Registry, patients with COVID-19 had a higher chance of developing severe illness in comparison to healthy people [49].

1.5.2.1.2 Subarachnoid Hemorrhage

The subarachnoid space is the area between the arachnoid layer and the pia mater which contains CSF. Bleeding into the subarachnoid space is a critical medical condition and is called subarachnoid hemorrhage. Although not very common, some cases of subarachnoid hemorrhage have been reported in COVID-19 patients [50][51].

1.5.2.1.3 Encephalopathy

Acute encephalopathy is acute impairment of brain function that is clinically present as an altered consciousness level [52]. Many forms of encephalopathy have been reported as clinical features of COVID-19 illness. It is interesting that encephalopathy could happen at the early stage of the illness or even as an initial symptom [53]. Several imaging methods can detect encephalopathy caused by COVID-19 illness including non-contrast CT scan and electroencephalography, which is the most commonly used technique.

1.5.2.1.4 Acute Hemorrhagic Necrotizing Encephalopathy

Acute necrotizing encephalopathy (ANE) is a type of encephalopathy that usually occurs after a febrile illness that is associated with a viral infection. Neurological manifestation of ANE includes multifocal symmetric lesions in the brain on CT scans or MR imaging [54]. ANE has been reported in COVID-19 patients with preliminary symptoms of cough, fever and altered mental status and the MRIs showed hyperintensity in bilateral medial temporal lobe and thalami [55].

1.5.2.1.5 Encephalitis

Acute viral encephalitis is a complication caused by a viral infection. Primary viral encephalitis is caused by the invasion and replication of the virus in the brain, while postinfectious encephalitis is mostly immune mediated [56]. The neurological complications of the disease include altered consciousness, confusion, hallucination,

aphasia, and abnormal movement. Hemorrhage, enhancement, or restriction of diffusion have been reported in MR images of such patients [57]. Encephalitis has been diagnosed in COVID-19 cases [44], [58], [59].

1.5.2.1.6 Meningitis/Encephalitis

Meningitis is the inflammation of the meninges which is the protective layer of the brain and spinal cord. Bacterial or viral infection of the CSF within the meningeal layer causes the inflammation. Meningitis and encephalitis have been reported in some severe COVID-19 patients [44], [60].

1.5.2.1.7 Demyelinating Disorders

Any condition that causes damage to the myelin sheath of the nervous system is called demyelination. Myelin is the protective layer surrounding nerves in the brain, optic nerve, and spinal cord. The damage can slow down or stop the impulses between nerves and cause neurological disorders. A wide range of demyelinating disorders have been reported in the literature in COVID-19 patients including Guillain-Barre Syndrome and Miller Fisher Syndrome that are autoimmune diseases where the immune system attacks the nerves and causes muscle weakness and paralysis in some cases. Although the first case of Guillain-Barre Syndrome associated with COVID-19 was not conclusive, over time, with more hospitalized COVID-19 patients in different studies the evidence has mounted. Male and elderly patients are the most affected group with acute, inflammatory, demyelinating poly-radiculomyelopathy [44], [61]. Miller Fisher Syndrome, a variant of Guillain-Barre Syndrome, has also been observed [62].

1.5.2.1.8 Central Nervous System Demyelination

Headache, anosmia, and dysgeusia are common symptoms of COVID-19 illness in the early stage of the disease. Brain MRI results confirmed newly demyelinating lesions in some COVID-19 patients [44], [63].

1.5.2.1.9 Seizures

Although seizures are not a direct effect of the infection in COVID-19 patients that have never had any previous brain injury or epilepsy, acute seizures are possible in some patients. The seizures have different factors such as cortical irritation as the result of blood brain barrier (BBB) breakdown due to the cytokine storm secondary to the viral infection [64]. Based on previous studies on SARS-coronaviruses, accumulation of pro-inflammatory cytokines such as interleukin-8 (IL8) and monocyte chemoattractant protein-1 (MCP1) could promote blood brain barrier breakdown. MCP1 is expressed in CNS cells and is transiently up-regulated during inflammation that cause BBB degradation [65]. Despite Epileptic seizures, non-Epileptic seizures (NES) are not caused by unusual electrical activity in the brain, and both have the same symptoms. A case of COVID-19 illness has been reported with NES as an initial symptom of the illness [66].

1.5.3 Neurological Side Effects Associated with COVID-19 Treatment

In the literature review by Al-Ramadan and colleagues, possible neurological side effects of different treatments of COVID-19 illness were reviewed. From the beginning of the pandemic, several treatments have been used to reduce the severity of the illness, reduce mortality, and reduce hospitalization [44]. Both the virus and the treatment might cause neurologic and psychiatric symptoms. Antiretroviral medications that are used to prevent replication of the virus may have CNS and PNS effects. These effects are different in severity and frequency based on the biological mechanism involved. For example, Lopinavir-Ritonavir combination could cause neurotoxicity despite its low penetration through the BBB. The combination could cause bilateral sensorineural hearing loss after 4 weeks of treatment with depressive symptoms [67].

Corticosteroids that inhibit immune responses to combat inflammation, could cause memory dysfunction and cognitive impairment due to the presence of a large number of corticosteroid receptors in the hippocampus [67]. Even high-dose short course corticosteroid treatments in COVID-19 patients may cause delirium and mood change

[68]. Although there is not clear evidence of any benefits associated with chloroquine, this drug has been used to stop the cytokine storm in COVID-19 patients to prevent acute respiratory distress syndrome (ARDS). The treatment can cause neuropsychiatric symptoms varying from mild (mood lability, nervousness) to severe (psychosis, suicidal tendency) and higher doses cause more severe complications [69].

1.5.4 Neuropsychiatric Complications of COVID-19 Illness

The high rate of psychiatric symptoms in acute COVID-19 patients is not surprising due to the physiological and psychosocial effects of the human coronavirus disease. Due to the CNS involvement of the virus and viral infection, psychiatric symptoms could contribute to neuropsychiatric complications [73]. It worth mentioning that antiviral medications that patients receive might also cause psychiatric problems. There is evidence that chloroquine and steroids can induce psychiatric episodes [74], [75]. The psychiatric complications observed in COVID-19 patients could be the result of all factors mentioned above [76].

Alteration in mental status is more common in patients with severe infection who need hospitalization especially those that need intensive care. These symptoms are prevalent in older patients and might reflect latent neurocognitive degenerative disease which relates to sepsis, hypoxia, and the use of different medications during treatment [77]. The alteration of acute mental status and primary psychiatric diagnoses such as psychosis were identified in a large group of COVID-19 patients [78]. Altered mental status included changes in personality, behavior, cognition, or consciousness. These findings cannot be extrapolated to patients with mild symptoms or people that were asymptomatic, but it gives robust prospective on severe patients that need hospitalization [77].

Neurological and psychiatric outcomes of COVOD-19 were assessed in a six-month retrospective cohort study [27]. The data showed that the incidence of neurological or psychiatric complications was 33.6% among survivors with 12.8% representing a new diagnosis. Most disorders were more common in severely ill patients, especially those who were hospitalized [27] and COVID-19 survivors were at higher risk of developing psychiatric issues [27], [79]. Approximately 43% of patients with neuropsychiatric

disorders had new onset of psychosis, 26% had neurocognitive (dementia-like) syndrome and 30% had other psychiatric disorder including catatonia and mania [77]. Experimental data have also shown that 43.1% of COVID-19 patients have depressive symptoms and 40.2% suffer from mental illnesses [80], [81]. Anxiety disorders, insomnia and dementia were also reported in COVID-19 patients [82]. Along the anxiety disorder spectrum, adjustment disorders, generalized anxiety disorders, post-traumatic stress disorder (PTSD) and panic disorder were common.

Patients hospitalized with COVID-19 infection with headache, anosmia, dysgeusia, diarrhea and those who needed oxygen therapy had lower scores in memory, attention and executive function subtests comparing to asymptomatic patients [83]. Patients who had headache and clinical hypoxia also had lower scores in global cognitive index. Patients with cognitive complaints at presentation had higher anxiety and depression [83].

Insomnia, aggressive behavior, delusion, and hallucinations have also been reported in COVID-19 patients in the literature [84]. Based on a meta-analysis of psychiatric symptoms of COVID-19 patients and survivors, almost all the psychiatric symptoms of the illness were severe during acute phase of the illness and then relieved to mild to medium during recovery, which suggest that acute stress reactions are the main psychiatric complications in the acute stage and is transient [76].

There is evidence that hospitalized patients who have recovered from COVID-19 illness still suffer from fatigue, muscle weakness, sleep difficulties, depression, and anxiety, even six months after acute infection [85]. Therefore, monitoring the psychiatric symptoms of COVID-19 patients after recovery and providing psychiatric consultations and treatments are of great importance [76]. Furthermore, based on the mental health continuum model, psychiatric symptoms may be early signs of mental disorders and the more severe and lingering the symptoms are the greater chance of developing mental disorders [86]. Consequently, timely diagnosis of mental health issues is vital in the clinical management of COVID-19 patients and survivors [76].

1.5.5 Post-Acute COVID-19 Syndrome

Post-acute COVID-19 syndrome describes lingering symptoms in patients after acute infection and recovery that are persistent and debilitating. These symptoms are not limited to hospitalized patients with severe acute infection. As reported by Tabacof *et al.*, patients who managed their illness without the need for hospitalization can also have post-acute symptoms, which are challenging for both patients and healthcare teams. The most prevalent persistent symptoms in the post-acute phase (more than 6 weeks after the onset of acute symptoms) are fatigue (92%), loss of concentration/memory (74%), weakness (68%), headache (65%), and dizziness (64%) [70]. These mainly neurological symptoms can be either persistent symptoms or new symptoms that emerge after recovery [44], [70]. In another study by Carfi *et al.*, persistent symptoms were assessed in discharged hospitalized patients around 60 days after the onset of the first COVID-19 symptoms. Only 12.6% of the patients did not report any COVID-19-related symptoms and 87% of patients reported persistence of at least one symptom with fatigue and dyspnea being the most common. It is worth mentioning that worsened quality of life was reported among 44% of patients [71]. Another prospective cohort study of recovered adult COVID-19 patients showed that half of COVID-19 survivors had post-acute syndrome 10-14 weeks after the onset of their symptoms. Radiological and spirometric alterations were observed in less than 25% of patients and were mild [72].

1.5.6 Brain Microstructural Changes in COVID-19 Patients

Magnetic resonance imaging (MRI) can be used to investigate brain structure, microstructure, and function following COVID-19 infection. Changes in micro-structural and functional integrity in recovered COVID-19 patients could suggest neuro-invasion of SARS-CoV-2 [8]. Micro-structural changes in the CNS can be detected by diffusion imaging methods, which may be more sensitive to tissue damage than gross structural measurements.

Previous MRI findings following COVID-19 infection have been varied. In one study, an enlarged volume of the central olfactory system including bilateral olfactory cortices and hippocampi was observed [8]. This study also found a decrease in some diffusion

tensor imaging (DTI) metrics (mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD)) accompanied by an increase of fractional anisotropy (FA) within white matter (WM) in recovered COVID-19 patients [8]. The decrease in MD values and increase in FA suggest a greater alignment of fibers and restricted diffusion occurs after infection [93]. The gray matter volumetric changes in the central olfactory system led to speculation that the SARS-CoV-2 virus might enter the CNS through a neuronal retrograde route [8]. The olfactory gyrus was recognized as the first functional area in the CNS to be infected by SARS-CoV-2 [94]. It has also been suggested that various limbic system components could be affected by infection due to a high ACE-2 expression [8]. Decreases in cortical thickness and cerebral blood flow (CBF) and changes in WM microstructures were found to be more severe in patients with severe illness compared to those with mild disease, especially in the frontal and limbic system and these changes were significantly correlated with inflammatory markers [10].

1.6 Thesis Objectives

The overall goal of this thesis was to determine the incidence of brain imaging abnormalities in COVID-19 survivors who experienced neurological symptoms and the association of these brain injuries with long-term cognitive and neuropsychological dysfunction. We performed an observational cohort study that examined patients after they recovered from COVID-19 illness using the highest magnetic field strength available in Canada for human brain MRI. Ultra-high field MRI increases sensitivity to measure cerebral microbleeds, cerebral vascular integrity, and brain microstructural abnormalities related to ischemic tissue damage.

We hypothesize that COVID-19 patients with neurological symptoms would have impaired cognitive function associated with the number of microbleeds in the brain, the presence of white matter hyperintensities, and tissue microstructural changes in subcortical brain regions. When initially conceived, the primary endpoint for this cohort study was focused on was the incidence of microbleeds and the secondary endpoints included assessments of diffusion abnormalities and white matter hyperintensities.

Understanding the impact of imaging changes in the brain on cognitive function could allow patients to be managed more effectively, increasing their quality of life, and relieving future impact on the healthcare system.

Chapter 2

2 Examining the Association between Brain MRI Measures at 7 Tesla and cognition following COVID-19 Infection

2.1 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes a range of multi-systemic symptoms and in severe cases can lead to respiratory failure and consequently death [120]. As the number of people infected with the coronavirus disease of 2019 (COVID-19) increases, it has become evident that some patients experience prolonged symptoms including fatigue, headache, weakness, dyspnea, sleep disturbance, and cognitive impairments, well beyond the resolution of respiratory symptoms. When symptoms last for more than 28 days after the initial onset of COVID-19 related symptoms, the condition is referred to as post-acute COVID-19 syndrome or long COVID [121]. Such longer-term complications have been observed in both hospitalized patients and non-hospitalized patients who experienced less severe forms of acute COVID-19 illness [122]. Long COVID symptoms may include several neurological symptoms suggesting effects on the brain either directly or indirectly [123]. The presence of the angiotensin converting enzyme-2 (ACE-2) receptor for the SARS-CoV-2 virus in the brain and brain stem also suggests the possibility of direct effects from viral invasion of the CNS [124]. However, a direct link between acute neurological symptoms and long-term changes in cognition and brain microstructure has not been established.

The SARS-CoV-2 virus activates the immune system of the host and can produce a cytokine response that leads to general inflammation [125]. Evidence also suggests that the virus generates a process of neuroinflammation [125], [126]. Inflammation within the brain can have both acute and long-term effects and may exacerbate neurodegenerative processes [83]. Interestingly, the limbic system and its related structures, including the hippocampi and basal ganglia, which are involved in cognitive processes such as memory, attention, emotion, and perception, contain more inflammatory related enzymes than do the primary motor or sensory cortices [127], [128]. Therefore, it is of particular

interest to examine changes in these structures following COVID-19 infection. COVID-19 infection may also be associated with a prothrombotic state and other coagulation disorders [129], [130]. Increased microbleeds have previously been reported in COVID-19 patients with severe symptoms usually requiring hospitalization [131], [132]. But it remains unclear if microhemorrhages are related to COVID-19 infection or a more general phenomenon associated with critical illness [133].

Several recent studies have highlighted the potential long-term health impact of COVID-19 illness including fatigue, anxiety, post-traumatic stress disorder (PTSD), and deficits in attention, mood, and memory [8], [16]– [19]. For example, in a systematic review and meta-analysis of approximately 48000 patients from 14 to 110 days after viral infection, the most common symptoms were fatigue (58%), headache (44%), attention disorder (27%), hair loss (25%), and dyspnea (24%) [134]. Another study examining impairments within 90 days of infection, found that the most common psychiatric disorders in COVID-19 survivors without previous cognitive impairments were anxiety disorders, including generalized anxiety disorders, post-traumatic stress disorder, and panic disorders [82]. Furthermore, patients with neurological symptoms including headache, anosmia, and dysgeusia had lower scores in memory, attention, and executive function subtests compared to asymptomatic patients [83]. Similarly, in hospitalized COVID-19 survivors without brain lesions, a systemic immune-inflammation index predicted worse depression and PTSD outcomes [135] almost 90 days after acute infection. Finally, survivors discharged from the hospital had higher PTSD scores using the diagnostic and statistical manual of mental disorders, fifth edition (DSM-5) [136] criteria than a control group and the score correlated with the duration after discharge [11].

Neuroimaging has shed light on the anatomical correlates of cognitive impairments in many neurological conditions [104]. Diffusion MRI specifically provides insight into white matter (WM) connectivity and overall tissue microstructural integrity [137]. Diffusion tensor imaging (DTI) which is an extended form of diffusion-weighted imaging, provides quantitative metrics that are sensitive to the movement of water within tissue microstructures [104], [138]. Specifically, fractional anisotropy (FA) and mean diffusivity (MD) are the two most common quantitative diffusion metrics. MD measures

the mean water diffusion and is typically increased when cellular structures break down resulting in an increase in free water diffusion [104]. FA measures anisotropic water diffusion and reflects the degree of directionality of cellular structures, particularly within white matter fiber tracts. However, the accuracy of FA values can be compromised in tissue regions with crossing fibers since this metric is sensitive to the degree of anisotropy as well as orientation dispersion [111]. More advanced diffusion MRI (dMRI) metrics can overcome this limitation. For example, microscopic fractional anisotropy (μFA) was recently developed to quantify water diffusion anisotropy independent of neuron fiber orientation [111], [139]. Similarly, diffusion kurtosis imaging (DKI) is an extension of DTI and is independent of the spatial direction of cellular structures [113]. Specifically, linear kurtosis (K_{lin}) is a non-specific parameter that is related to the heterogeneity in the size and shape of cells [111]. Several previous studies have examined DTI metrics using 3 Tesla MRI in sub-cortical structures in healthy individuals [140] and different neurological diseases including Progressive Supranuclear Palsy [141], Tourette syndrome [142], Parkinson's disease [143], and amyotrophic lateral sclerosis [144] among many others. However, μFA is a very recently developed measurement that has not yet been applied to study any of the above diseases or COVID-19. Although the majority of brain imaging studies following COVID-19 infection have been performed on conventional 1.5T or 3T MRI scanners [8], [11], [123], [145], [146], the use of ultra-high field MRI (UHF-MRI) at magnetic fields ≥ 7T provides greater sensitivity to microbleeds and higher signal-to-noise ratio (SNR) [97], [147], [148]. The advantages of high-field MRI translate into images with greater resolution and contrast across a wide range of neurologic disorders and psychiatric conditions [149]. UHF-MRI (e.g., 7T) has previously been used to provide a comprehensive assessment of DTI and DKI parameters in neurological conditions like amyotrophic lateral sclerosis (ALS) [150], however, 7T MRI has yet to be applied to study brain changes following COVID-19 infection.

The long-term cognitive and neuropsychological effects of COVID-19 illness in survivors with mild symptoms are still poorly understood. The current study examined cognitive and neuropsychological changes approximately two months after infection and the association of changes with advanced dMRI correlates within subcortical brain

structures of the limbic system using 7T MRI. Specifically, we examined whether specific cognitive, psychological, and diffusion metrics were linked to neurological symptoms experienced during the acute phase of illness. We hypothesized that COVID-19 survivors who experienced acute neurological symptoms would show impairments in cognitive function associated with an increased number of microbleeds in the brain and evidence of tissue microstructure damage within structures of the limbic system.

2.2 Materials and Methods

2.2.1 Study Design

This study was approved by the Western University Health Sciences Research Ethics Board. This observational cohort study examined people who had recovered from the respiratory symptoms of COVID-19 illness and had neurological symptoms during the acute phase of illness. There was no randomization for this study. During a baseline visit, conducted either in person or over the phone depending on COVID-19 restrictions, verbal or written consent was obtained from each participant and demographic information, medical/surgical history, and medications were documented.

The study included three separate additional sessions (Figure 2-1). Briefly, the second session included two over-the-phone cognitive tests; the Telephone Interview for Cognitive Status (TICS) and the telephone-based Montreal Cognitive Assessment (MoCA-Blind). For the third visit, participants went to the Robarts Research Institute at Western University to complete neuropsychological and cognitive testing as well as a 7T MRI scan. Cognitive testing was performed using the comprehensive Repeatable Battery for Neuropsychological Status (RBANS) test administered by study staff [114], while neuropsychological data were gathered using the patient-reported outcomes measurement information system (PROMIS) American Psychiatric Association Assessment Measures (APA), based on Section III of the *DSM*-5 self-report questionnaire [151]. The Columbia Suicide Severity Rating Scale (CSSRS), a suicidal ideation and behavior rating scale, was also administered to evaluate suicidal risk [152]. Patients were asked if they had any previous head injuries. A brain MRI scan without contrast was performed on a 7T MRI at Western's Centre for Functional and Metabolic Mapping. The

fourth and final 6-month follow-up assessment was performed by phone and included the TICS and MoCA-Blind scales. An adverse event report was collected in each visit. Research staff and participants abided by all COVID-19 infection public health prevention requirements throughout the study.

Figure 2-1: Study Procedures and Schedule of Events

2.2.2 Participants

Sixty-three patients met the inclusion/exclusion criteria and were recruited from southwestern Ontario between September 2020 and December 2021 at the multidisciplinary virtual London Health Sciences Centre Urgent COVID-19 Care Clinic (LUC3) in London, Ontario. The LUC3 clinic was designed to care for high-risk patients with polymerase chain reaction (PCR) confirmed COVID-19. These patients were referred to the clinic from the local public health unit, emergency department, recent hospital admission, or from family physicians. High-risk patients included those over the age of 40 and/or those over the age of 18 with pre-existing medical conditions (e.g., chronic respiratory illness) putting them at risk of hospitalization with severe COVID. Study participants who reported a heavy burden of any neurologic symptom (headaches, brain fog, anosmia, paraesthesia, etc.) were approached about participation in the study.

Included patients were aged from 18 to 85 years with a confirmed diagnosis of COVID-19 within the past six months. Participants must have had neurological symptoms during the acute phase of illness and were free of COVID-19 respiratory symptoms for at least one month. Participants were excluded if they had current respiratory symptoms consistent with COVID-19 or if they have evidence of acute psychosis, pre-existing dementia, or previous cognitive impairments. Subjects with contraindications to 7T MRI (e.g., metal implants, claustrophobia, inability to lie still in the scanner, and pregnant or breastfeeding women) were also excluded.

2.2.3 Neuropsychological Assessments

To assess long-term cognitive and neuropsychological impairments, a set of tests and questionnaires were administered. The tests included the Repeatable Battery for Neuropsychological Status (RBANS) with 12 subtests that measured five different indices: the immediate memory index (list memory and story memory subtests), the delayed memory index (list recall, list recognition, story recall, and figure recall subtests), the visuospatial/constructional ability index (figure copy and line orientation subtests), the attention index (digit span and coding subtests), and the language index (picture naming and semantic fluency subtests) [114]. The raw scores from the RBANS subtests were transformed into index scores according to the RBANS manual [114]. The RBANS total scale was calculated from the score of these five index scores. The index scores range from 40 to 160 within the age-adjusted normative data with a mean of 100 for each index score and a SD of 15, where lower scores represent worse performance [114]. To assess if neurocognitive status was impaired the procedure suggested by Girard et al [153] and adapted for RBANS by Mitchell et al [154] was applied [146]. A trained examiner administered the RBANS, which took approximately 20-30 minutes. For the current study, participants were excluded from cognitive test analysis if English was not their first language, because there are no normative values for people with English as their second language. Participants then completed the American Psychiatric Association assessment, DSM-5 PROMIS questionnaires that measure cross-cutting symptoms: somatic symptoms (total raw score), sleep disturbance (T-score), depression (T-score), anxiety (T-score), anger (T-score), PTSD, and acute stress disorder (average total score)

[151]. Also, the Columbia Suicide severity rating scale (CSSRS) was administered by the trained examiner after the DSM-5 measures [152]. All the tests were performed the same day and prior to the MRI. This testing session lasted approximately one hour.

2.2.4 Magnetic Resonance Imaging

Brain magnetic resonance imaging was performed on a Siemens Magnetom 7T Plus MRI scanner with a head-only gradient coil (80 mT/m strength and 400 T/m/s slew rate) and a customized 8-channel transmit and 32-channel receive radio frequency (RF) coil following the cognitive assessments as part of Visit 3. The MRI protocols (parameters provided in Table 2-1) included high resolution anatomical T_1-weighted MP2RAGE (700 μm isotropic), fluid attenuated inversion recovery (FLAIR, 800 μm isotropic), 3D time of flight imaging (470 μm isotropic), gradient echo (GRE) imaging to assess microbleeds (0.1x0.1x1.3 mm^3), and diffusion MRI (including μFA, 2mm isotropic). MRI scans were assessed by a blinded expert neuroradiologist to identify anomalies including microbleeds, white matter hyperintensities, and other clinically relevant findings.

Table 2-1 : 7T MRI Protocols and acquisition parameters

MRI Sequence	Parameters	Scan Time (min:s)
3D-magnetization-prepared rapid gradient echo (MP2RAGE)	TR/TE=6000/2.74 ms; TI_1=800 ms; TI_2=2700 ms; θ_1= 4°; θ_2= 5°; 0.7-mm isotropic voxels	10:12
3D-T_2-weighted fluid-attenuated inversion recovery (FLAIR)	TR/TE/TI=9000/268/2600 ms; 0.8-mm isotropic voxels	8:17
3D-multislice gradient echo (GRE)	TR/TE=21/14 ms; Acceleration factor=3; 80 slices; 30% oversampling; 0.1x0.1x1.3mm voxels	8:06
3D-diffusion-tensor imaging (DTI)	TR/TE=6400/91 ms; 2-mm isotropic voxels; 66 slices; acceleration factor=2; Shell1: b-value 1000 s/mm^2, Shell 2: b-value 2000 s/mm^2	9:38
3D-time of flight-angiography (TOF)	TR/TE=12/3.59 ms; Acceleration factor=3; 18.2% oversampling; 44 slices; 0.5-mm isotropic voxels	6:13

Overall	42:26

TR ≡ repetition time, TE ≡ echo time, TI ≡ inversion time, θ ≡ flip angle

2.2.5 Diffusion MRI

2.2.5.1 Image Acquisition

The diffusion MRI (dMRI) sequence (Table 2-1) included linear and spherical b-tensor encoding in the same acquisition, similar to previous work [108]. Linear encoding was acquired in 2 shells, using 9 directions with b-value 1,000 s/mm^2 and 24 directions with b-value 2,000 s/mm^2. Forty-two spherically encoded images were acquired; 30 images with a b-value of 2,000 s/mm^2 and 12 images with a b-value of 1,000 s/mm^2. Five b-values = 0 images and a reverse phase encoding b-value = 0 image were also acquired for frequency drift and distortion correction respectively.

2.2.5.2 Processing

The raw diffusion data were corrected for Gibb's ringing [155] and noise (via PCA denoising) using the matrix3 package [156] , and eddy current [157], and distortion [158] using the FSL package. The traditional diffusion metrics (FA and MD) were extracted using the mrtrix3 package [159]. Finally, μFA was calculated by combining the linear fit of the power averaged signal from linear and spherical encoding up to the second term of the cumulant expansion [111].

2.2.5.3 Segmentation of Sub-Cortical Structures

Using the anatomical T_1-weighted MP2RAGE images, the brain was extracted using the FMRIB Software Library (FSL, Version 6) [160], and subcortical brain structures were segmented [161]. Seven subcortical structures were segmented, including left and right caudate, putamen, pallidum, hippocampus, accumbens, amygdala, and thalamus (Figure 2-2). FSL was used to measure the volume of each of the mentioned subcortical structures.

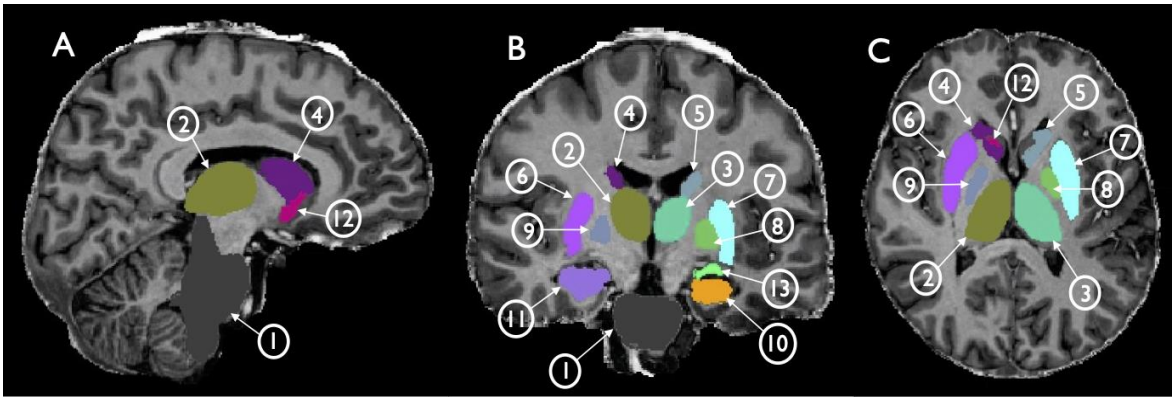

Figure 2-2: T1-weighted anatomical MP2RAGE images showing the subcortical regions included in the current study: A: sagittal view, B: coronal view, C: axial view. 1: Brain stem; 2: right thalamus; 3: left thalamus; 4: right caudate; 5: left caudate; 6: right putamen; 7: left putamen; 8: right pallidum; 9: left pallidum; 10: left hippocampus; 11: right hippocampus; 12: right accumbens; 13: left amygdala.

The segmentation results were visually verified for each subject by overlaying the segmented subcortical structures on the T_1-weighted MP2RAGE brain extracted images in the FSL viewer (Version 6). To create a mask of each structure that minimized the inclusion of pixels with partial volume artifact around the edges, all structure edges were eroded using a cube kernel in MATLAB (Version R2019b Update 3, The MathWorks, Inc., USA). Based on the voxel counts, a threshold of 2000 voxels were used to apply either a one voxel or two voxel erosion. Both the accumbens and amygdala contained <2000 voxels and consequently were eroded by one voxel while all other subcortical structures were eroded by two voxels (Figure 2-3).

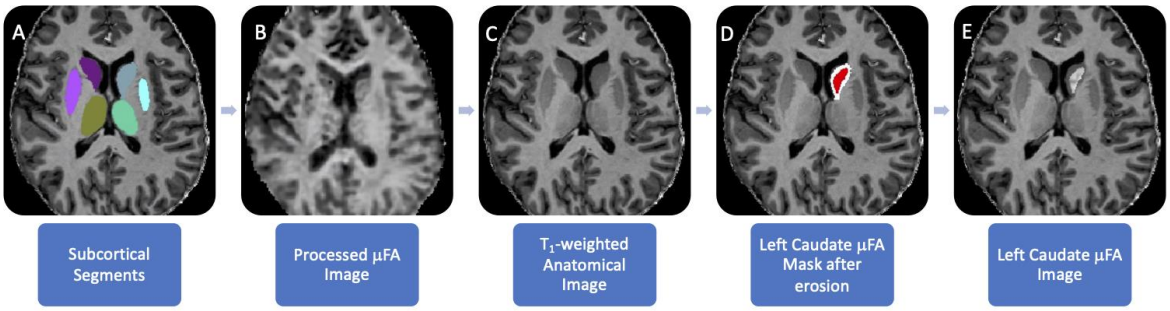

Figure 2-3: Description of the subcortical segmentation pipeline highlighting μFA diffusion MRI analysis within the caudate. A: T_1-weighted axial image with subcortical structures shown with colors corresponding to Figure 2. B: Corresponding μFA map. C: The corresponding T_1-weighted anatomical image. D: Left caudate mask (white pixels) and left caudate mask after 2 voxels erosion to minimize partial volume effect (red pixels). E: the final left caudate mask used to calculate mean μFA within the left caudate, superimposed on the T_1-weighted anatomical image.

The diffusion-weighted images (including maps of μFA, FA, MD, K_{lin}) were registered to the T_1-weighted anatomical MP2RAGE images using an affine registration (FLIRT) in FSL. FLIRT is a fully automated, robust, and accurate tool for linear (affine) intra- and inter-modal brain image registration with improved EPI to structural registration and distortion-correction [162]. The eroded segments were used as masks to identify pixels within each structure in the diffusion maps. All pixels contributing to a structure were used to measure the mean, median, and standard deviation (SD) of each diffusion metric within each sub-cortical region.

2.2.6 Statistical analysis

SPSS Statistics (V.27, International Business Machines, USA) was used for all analyses. Descriptive statistics were generated to provide the minimum, maximum, mean, and SD for the RBANS indices and the DSM-5 measures. A one-sample *t*-test (p-value<0.05 was

considered significant) was performed to determine whether the mean values of the participants performance for the six RBANS indices significantly differed from the mean performance of the normative US population within the Battery (mean 100). The impact of the neurological symptoms present during the acute phase of illness on long-term cognitive performance and diffusion metrics was assessed by grouping participants into two group: those with and those without specific symptoms. Comparisons were only made for symptoms if groups contained at least ten subjects. Dependent variable values were generally not normally distributed. Therefore, independent non-parametric Mann Whitney U-tests were used to compare metrics between groups. Each diffusion metric and symptom were considered a separate analysis because different participants were included in the groupings of those with and without symptoms. A Bonferroni correction was used to reduce the chance of a Type 1 error for each analysis due to the inclusion of fourteen different regions (left and right for 7 brain regions). Therefore, the Bonferroni adjusted *p*-value for this analysis was 0.05/14 = 0.0036. The normality of the data was assessed using the Kolmogorov-Smirnov test. Non-parametric Spearman's correlation tests were used to assess associations between RBANS indices and diffusion metrics. Since age is an important factor that can affect brain structure volumes, the relationship between brain structure volumes and cognitive and diffusion metrics was assessed using a partial correlation analysis, with age as a covariate. All statistical tests were two-sided. For the correlation analyses, *p*-values <0.05 were considered significant.

2.3 Results

2.3.1 Demographic and Clinical Characteristics

Sixty-three participants who tested positive for SARS-CoV-2 were recruited between September 2020 and December 2021. The MRI and neuropsychological testing at Visit 3 were performed with mean (138±54) days from the onset of initial symptoms. Three participants were excluded: one had MRI incompatible eyebrow microblading, one had an MRI incompatible ear prosthetic, and one had a wire in their jaw. In addition, thirteen participants did not complete the MRI portion of the study and were excluded from imaging analyses: one participant did not fit into the scanner, four participants were claustrophobic, and eight participants withdrew themselves from the study after the first

phone visit. Two additional participants were excluded from diffusion MRI analyses because these data were not acquired. Of the fifty-one patients who completed RBANS (the four patients that were claustrophobic completed the RBANS but not the MRI), eight spoke English as their second language (ESL) and were excluded from the analysis of RBANS and other cognitive assessments. Medical histories of each participant were collected at the first visit. Of the 45 participants included in the imaging analysis and the 43 participants included in the cognitive assessments, the mean age was (45 ± 16) years, and 76% were female.

In this study cohort, one patient (2%) required treatment in the intensive care unit (ICU), two participants (4%) required oxygen (hypoxia), two participants (4%) had COVID-related pneumonia hospitalization, and one participant (2%) was hospitalized due to a pulmonary embolism. The average hospital stay for these participants was seven days. However, overall, the study cohort was predominantly composed of those labelled with mild COVID illness. The clinical and demographic characteristics of the studied population are presented in Table 2-2.

Table 2-2 : Clinical Symptom Frequency

Symptom		Category	Percent	Males	Females	Mean Age
Headache		Neuro	93	12	32	44.5
Brain fog		Neuro	93	12	31	43.9
Change/loss of taste or smell	*	Neuro	69	09	23	45.9
Confusion	*	Neuro	44	06	15	48.4
Fever	*	Neuro	62	10	19	45.4
Chills	*	Neuro	33	03	13	42.7
Peripheral neuropathy	*	Neuro	29	04	09	42.9
Anxiety	*	Psych	62	06	23	45.2
Fatigue	*	Psych	93	12	32	43.9
Decreased mood	*	Psych	49	06	16	42.5
Acute Stress Disorders	*	Psych	51	03	20	42.8
PTSD[a]	*	Psych	56	04	22	40.6
Nausea	*	GI	56	07	20	46.2
Diarrhea	*	GI	58	10	18	48.0
Vomiting	*	GI	27	02	10	45.9

Abdominal Pain	*	GI	36	08	09	53.5
Decreased appetite/oral intake	*	GI	53	09	16	42.8
Dyspnea		Resp	82	10	30	46.4
Cough	*	Resp	71	10	24	48.6
Sore throat		Resp	20	05	06	38.0
Nasal congestion	*	Resp	38	02	16	39.9
Asthma		Resp	16	00	08	45.4
WM[b] hyperintensity		Imaging	53	08	17	48.7
Microbleeds		Imaging	11	02	03	37.6

[a] Post-Traumatic Stress Disorders
[b] white matter
* Indicates a symptom included in group analysis of diffusion MRI measures

The most common neurological symptoms (Table 2-2) experienced during the acute phase of the illness in study participants were brain fog (93%), headache (93%), change/loss of taste or smell (69%), fever (62%), and confusion (44%). The most frequent non-neurological symptoms (Table 2-2) included fatigue (93%), anxiety (62%), PTSD (56%), decreased mood (49%), and acute stress disorder (51%).

2.3.2 MRI Clinical Interpretation

Forty-five participants completed the MRI scan. All scans were read by a blinded neuroradiologist (Dr. Michael Jurkiewicz) and findings are summarized in Table 2-3. Eleven participants (24%, mean age of 55 years) showed abnormalities on the 3D T_1-weighted anatomical images that were possibly related to COVID infection. The most common anatomical finding was supratentorial volume loss in six (13%) participants. Patchy FLAIR hyperintensities were reported in 25 participants (53%), mainly in the bilateral cerebral white matter: 4 participants (9%, mean age of 66 years) showed chronic small vessels ischemic disease (SVID); 8 participants (18%, mean age of 60 years) showed changes that were most likely SVID, and 7 participants (16%) had hyperintensities in the bifrontal cerebral white matter, most commonly observed in individuals with migraine. Of these participants only one patient had a previous clinical history of chronic migraines. Four participants (9.3%) had foci of susceptibility effect on the GRE images. Figures 2-4A and 2-4B shows examples of abnormally increased

susceptibility in the GRE images of younger patients and Figures 2-4C, 2-4D, and 2-4E show examples of FLAIR hyperintensities observed in three younger patients.

Table 2-3 : MRI Findings and Frequency

Findings	Percent	Frequency	Mean Age[f]
T_1-weighted			
Supratentorial volume loss	13.3	6	64
Hypo-intensity along right ventricle	2.2	1	50-54
Hyperintensity on left thalamus	2.2	1	45-49
GM[a] heterotopia along right ventricle	2.2	1	55-59
Focal outpouching from lateral ventricle	2.2	1	55-59
Developmental venous anomalies in anterior right and left frontal lobe	2.2	1	35-39
FLAIR[b]			
Chronic SVID[c]	8.9	4	66.3
Hyperintensities in bilateral cerebral WM most likely SVID	17.8	8	60.4
Hyperintensities in bilateral cerebral WM, SVID or migraines	4.4	2	55.0
Hyperintensities in bilateral cerebral WM with frontal predominance, most likely migraines	15.6	7	36.8
Hyperintensities in periventricular WM, demyelination disease and ischemic	2.2	1	45-49
Single hyperintensity in deep frontal left WM	2.2	1	35-39
SWI[d]			
Developmental venous anomalies in frontal lobe	4.4	2	44.5
Abnormal susceptibility in left caudate head	2.2	1	60-64
Abnormal susceptibility in left superior temporal lobe	2.2	1	35-39
Abnormal susceptibility in right precentral gyrus	2.2	1	60-64
Abnormal susceptibility in left cerebral hemisphere	2.2	1	50-54
3D-TOF[e]			
Aneurysm	4.4	2	65.0

[a] gray matter
[b] fluid attenuation inversion recovery
[c] small vessels ischemic disease
[d] susceptibility weighted imaging
[e] 3D- time of flight angiography
[f] For Frequency=1, an age range is provided to maintain anonymity

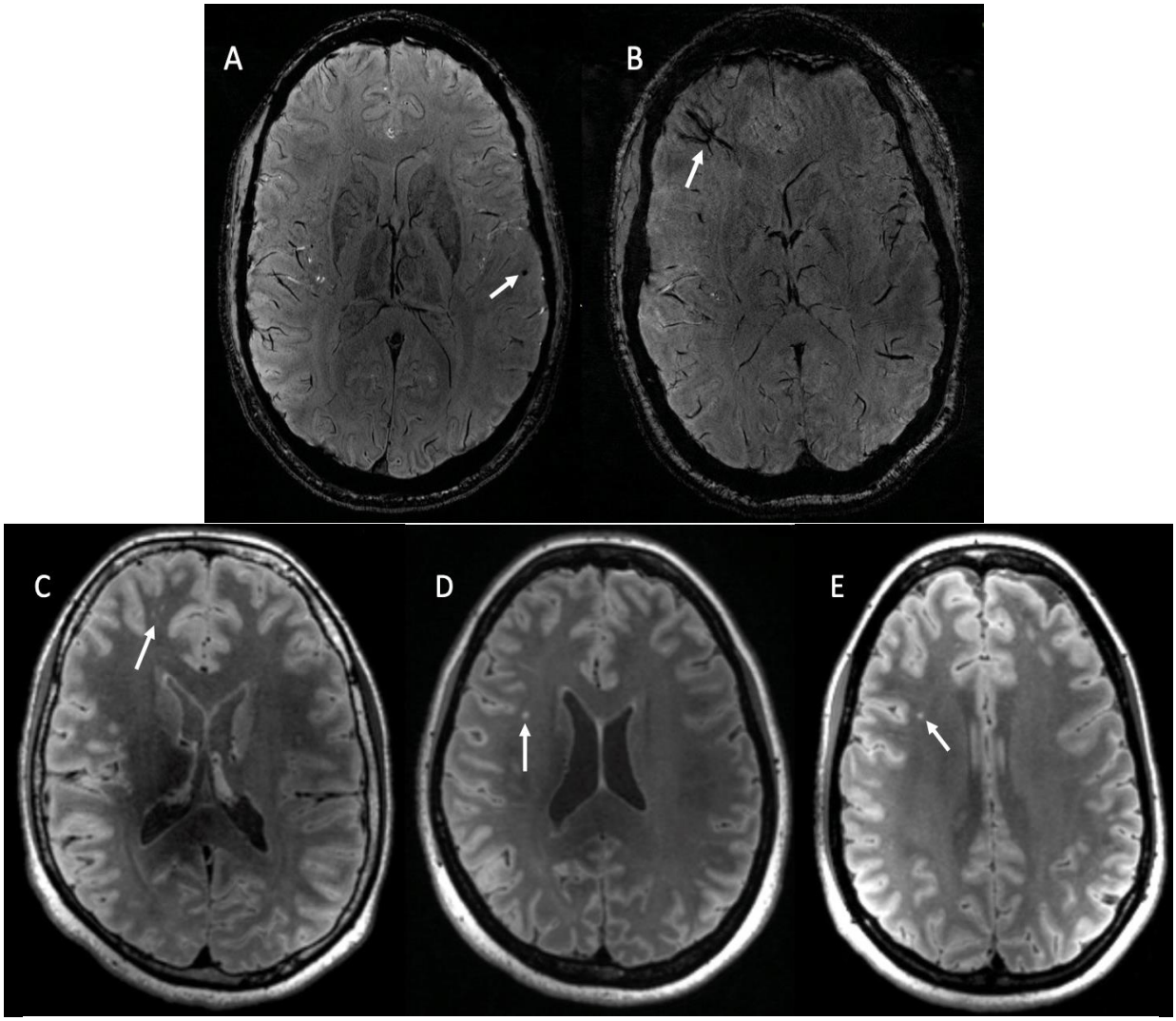

Figure 2-4: Examples of WM hyperintensity and abnormal susceptibility. A: Axial GRE (slice thickness 1.25mm) of a 35-39 year old man with a punctate focus abnormal susceptibility on the left superior temporal gyrus; **B**: Axial GRE (slice thickness 1.25mm) of a 35-39 year old woman with a developmental venous anomaly in the right anterior frontal lobe; **C**: Axial FLAIR image (slice thickness 0.8mm) of an 18-24 year old woman **D**: Axial FLAIR image (slice thickness 0.8mm) of a 35-39 year old woman; **E**: Axial FLAIR image (slice thickness 0.8mm) of a 30-34 year old woman. All three FLAIR images show nonspecific foci of hyperintensities in the bilateral cerebral WM with frontal lobe predominance commonly seen in patients with migraines. Age ranges are provided to maintain anonymity.

2.3.3 Cognitive and Neuropsychological Evaluation

Forty-three patients were included in the assessment of cognitive performance and neuropsychological evaluation. Descriptive statistics of the cognitive testing performed in this cohort, including the DSM-5 PROMIS measures reported as T-scores and the RBANS index scores are provided in Table 2-4.

Table 2-4 : Neuropsychological Characteristics

Characteristic	Minimum	Maximum	Mean ±S.D	*p*-Value[c]	*t*-Value[d]
Age	18.0	75	45.2±15.9		
RBANS[a] index					
Immediate Memory	73.0	126	100.4±13.9	0.853	0.186
Visuospatial/Constructional	60.0	126	96.0±16.7	0.130	-1.543
Language	79.0	127	100.9±11.0	0.612	0.511
Attention	60.0	122	93.9±14.4	0.008*	-2.773
Delayed Memory	48.0	125	98.7±14.6	0.548	-0.606
Total Scale	66.0	121	97.0±12.7	0.128	-1.554
DSM-5[b] T-score					
Level 2-Somatic Symptoms	01.0	22	10.5±5.4		
Level 2-Sleep Disturbance	35.9	76.5	57.5±9.0		
Level 2-Depression	37.1	81.1	51.4±11.2		
Level 2-Anxiety	36.3	75.8	54.0±11.0		
Level 2-Anger	32.9	83.3	51.3±11.0		
PTSD (NSESSS)	00.0	03	0.90±1.0		
Acute Stress Disorder (NSESSS)	00.0	03	0.90±0.80		
Days between symptoms onsets and cognitive assessments/MRI	53.0	252	139.5±54.4		

[a] Repeatable Battery for the Assessment of Neuropsychological Status
[b] Diagnostic and Statistical Manual of Mental Disorders,5th edition
[c, d] Comparison of RBANS (one sample *t*-test) to normative data with mean = 100, *df*=42
* $p<0.05$

A one-sample *t*-test was used to compare the mean performance of the studied population across the six RBANS indices with the standard mean performance of the normative population (100±15) (Table 2-4). The group performed significantly worse in the attention domain relative to the normative population (p=0.008). There was no significant

correlation between the time interval between the onset of acute symptoms and cognitive testing and the RBANS index scores. However, there was a moderate correlation between education and immediate memory index score (p=0.002, R=0.45) and delayed memory index score (p=0.014, R=0.37) (Table 2-5). Overall, according to Mitchell's definition for RBANS [154], 81% of the population showed minimal cognitive impairments, 14% were mildly/moderately cognitively impaired, and 5% were severely cognitively impaired. (Table 6)

Table 2-5 : Correlations between RBANS Indices and Demographic Variables

RBANS Index	Education		Days between symptoms onset and RBANS Administration	
	***p*-Value**	**R-Value**	***p*-Value**	**R-Value**
Immediate Memory	0.002*	0.451	0.769	0.046
Visuospatial/Constructional	0.520	0.101	0.107	0.250
Language	0.051	0.299	0.330	0.152
Attention	0.857	-0.028	0.065	0.284
Delayed Memory	0.014*	0.371	0.357	0.144
Total Scale	0.030	0.332	0.090	0.261

* P<0.05, Spearman's Rho

Table 2-6 : Severity of Neuropsychological Symptoms

Neuropsychology Assessments	Minimal	Mild/Moderate	Severe
RBANS	81.4%	13.9%	4.6%
DSM-5 Somatic symptoms	7.8%	60.8%	31.4%
DSM-5 Sleep disturbance	41.2%	47.0%	1.8%
DSM-5 Depression	62.8%	29.4%	7.8%
DSM-5 Anxiety	47.0%	43.1%	9.8%
DSM-5 Anger	70.6%	25.5%	3.9%
NSESSS PTSD	41.2%	47.0%	11.8%
NSESSS Acute stress disorder	45.1%	47.0%	7.8%

The severity of various neuropsychological disturbances is summarized in Table 2-6 based on the DSM-5 measures [151]. Interestingly, the assessments showing the most severe dysfunction were somatic symptoms (31.4% of the cohort), PTSD (11.8% of the cohort), and anxiety (9.8% of the cohort). Similarly, the assessments showing the greatest

prevalence of mild/moderate dysfunction were somatic symptoms (60.8% of the cohort), PTSD (47% of the cohort), acute stress disorder (47% of the cohort), and sleep disturbance (47% of the cohort). Depression and anger scores were least likely to be affected with anger affecting 70.6% of the cohort minimally, and depression affecting 62.8% of the cohort minimally. Seven (16.3%) participants reported suicidal ideation with no imminent plan or intent, mainly during their acute illness according to CSSRS measures [152], and were referred for psychological services.

Patients were grouped based on the presence or absence of the symptoms reported during the acute phase of their illness. Comparisons between neuropsychological measures, cognitive measures, and imaging metrics were made between groups. To ensure a sufficient number of participants were included in each group, only clinical variables with $N \geq 10$ per group and a prevalence of 25%-75% were included in the analyses. For this reason, headache, brain fog, fatigue, and dyspnea (Table 2-2) were excluded from these analyses since most patients reported these symptoms, and sore throat and asthma were present in less than 25% of the population.

Non-parametric Mann-Whitney *U*-tests were used to compare the mean values of different cognitive and neuropsychological measures between the groups of participants with and without specific symptoms (Table 2-7). Participants with PTSD (56%) had a significantly ($p<0.01$) worse depression, anxiety, anger, and acute stress disorder DSM-5 scores than those without PTSD. Decreased mood was reported in 49% of the sample and these participants also had significantly higher depression score compared to the group without the symptom. Participants with nausea (56%) had a higher score in the DSM-5 somatic symptoms. The RBANS language index score was lower in the patients having diarrhea (58%) compared to those without, and subjects with decreased appetite/oral intake (53%) had significantly lower scores in RBANS visuospatial/constructional abilities and total score. These cognitive findings remained significant after excluding the six patients that required hospitalization during their acute illness.

Table 2-7: Significant Neuropsychological Findings Related to Clinical Characteristics

Symptoms / Subtests	*Z*-Value	*p*-Value	Mean With	Mean Without
PTSD (N[a]=26)				
DSM-5 Depression	-3.16	0.002*	56.8	45.5
DSM-5 Anxiety	-3.22	0.001*	58.5	48.1
DSM-5 Anger	-2.60	0.009*	55.4	46.4
Acute Stress Disorder	-4.92	<0.001*	01.5	0.05
Decreased mood (N=22)				
DSM-5 Depression	-2.62	0.009*	56.0	47.7
Nausea (N=27)				
DSM-5 Somatic Symptoms	-3.09	0.002*	12.6	8.0
Diarrhea (N=28)				
RBANS LGI[b]	-2.78	0.005*	90.3	106.0
Decreased appetite/oral Intake (N=25)				
RBANS VCI[c]	-3.41	<0.001*	89.1	106.0
RBANS TS[d]	-2.69	0.007*	92.4	100.7

[a] N refers to the numbers having the symptom
[b] Language Index
[c] Visuospatial/Construction Index
[d] Total Score Index
* $P<0.05$, independent Mann-Whitney U-test

2.3.4 MRI Diffusion Changes Associated with Cognitive Performance

Non-parametric partial correlation with age as a covariate was used to examine associations between diffusion metrics in subcortical structures, subcortical structure volumes, and cognitive assessments. Although significant correlations were detected with some RBANS indices, the R-values were low (Table 2-8). Therefore, no strong correlations were observed between diffusion measures and cognitive assessments.

Table 2-8 : Correlation between RBANS and Diffusion Metrics

Subcortical Structure	RBANS Index	*p* -Value	*R*-Value
Hippocampus			
L[a]-Hipp-MD	Attention	0.037*	-0.331
	Total Scale	0.029*	-0.345
Thalamus			
L-Thal-FA	Language	0.013*	0.391
L-Thal-K_{lin}	Language	0.037*	0.330
R[b]-Thal-K_{lin}	Language	0.019*	0.368
Accumbens			
L-Accu-μFA	Immediate Memory	0.035*	0.334

[a] Left
[b] Right
* $P<0.05$, partial correlation with age used as a covariate

2.3.5 MRI Diffusion Differences Associated with Neurological Symptoms

Subcortical volumetric analysis showed a moderate correlation between left and right caudate volume, left thalamus volume, and left and right putamen volume and age (Table 2-9). However, there were no differences in subcortical structure volumes when grouping participants by the presence of symptoms.

Table 2-9 : Correlation Between Age and Subcortical Volume

Subcortical Structure Volume	Age	
	***p*-Value**	***R*-Value**
L-Caud-Volume	0.003*	-0.458
R-Caud-Volume	0.003*	-0.448
L-Thal-Volume	0.004*	-0.435
L-Puta-Volume	0.008*	-0.411
R-Puta-Volume	0.005*	-0.431

* $P<0.01$, Spearman's Rho

Non-parametric Mann-Whitney *U*-tests revealed several differences in advanced diffusion measures within subcortical structures when comparing groups with and

without symptoms (Table 2-10). Considering the caudate nucleus, we found higher FA associated with diarrhea. In the thalamus, lower MD was associated with chills. In the putamen, higher K_{lin} were associated with diarrhea. Higher K_{lin} and FA were associated with nasal congestion in the pallidum. In the amygdala, higher μFA was associated with nasal congestion and in the accumbens nucleus lower FA was related to cough (Figure 2-5).

Table 2-10 : Diffusion MRI findings related to clinical characteristics

Subcortical Structure	Symptom	N	*Z*-Value	*p*-Value	Mean With	Mean Without
Caudate						
R-Caud-FA	Diarrhea	26	-3.03	0.002*	0.40	0.33
Thalamus						
L-Thal-MD	Chills	15	-3.35	<0.001*	0.00080	0.00088
Putamen						
L-Puta-K_{lin}	Diarrhea	26	-3.63	<0.001*	1.22	1.04
Pallidum						
R-Pall-FA	Nasal Congestion	17	-3.06	0.002*	0.89	0.81
R-Pall-K_{lin}	Nasal Congestion	17	-3.46	<0.001*	2.05	1.77
Amygdala						
L-Amyg-μFA	Nasal Congestion	17	-4.03	<0.001*	0.54	0.37
Accumbens						
L-Accu-FA	Cough	32	-3.23	0.001*	0.38	0.55

* $p<0.01$, Independent samples Mann-Whitney *U*-test

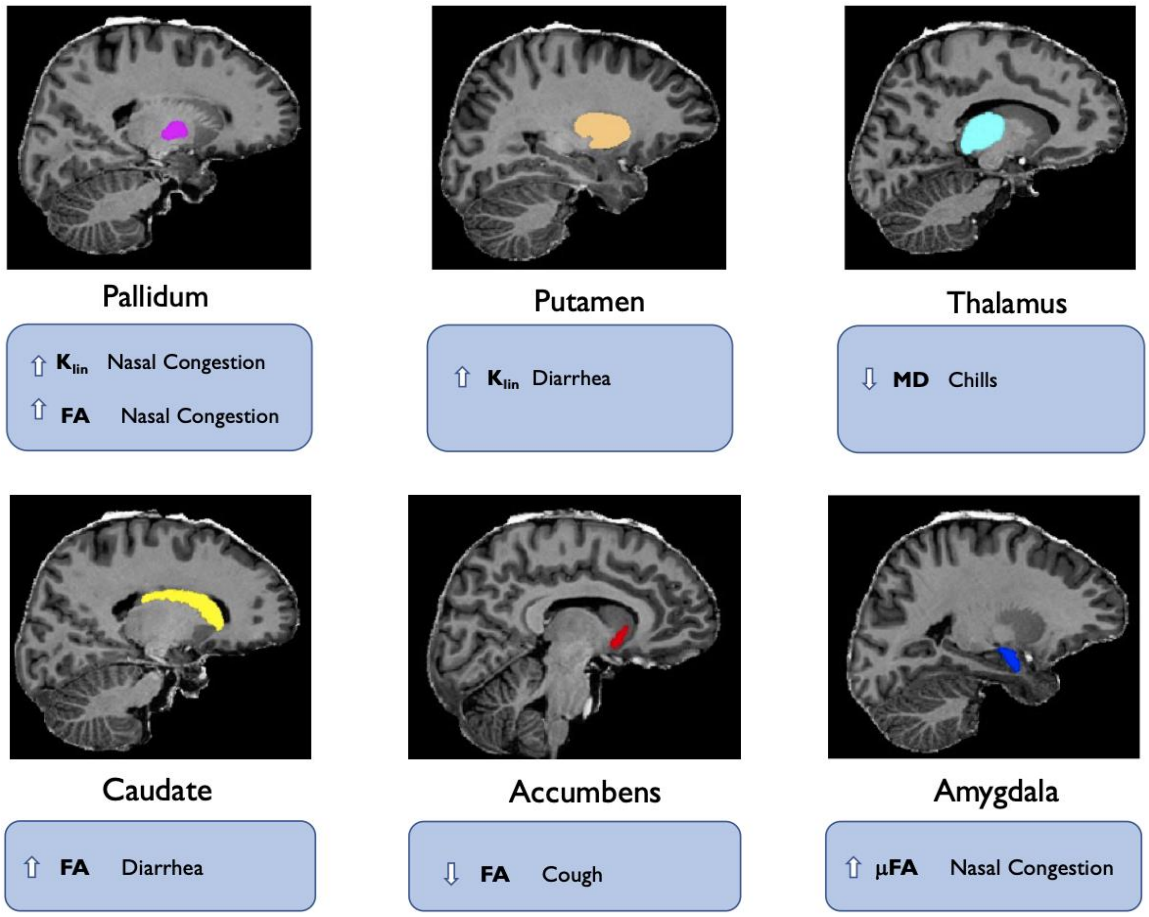

Figure 2-5: Significant diffusion MRI findings in subcortical structures related to symptoms present at the time of acute illness

There was a negative moderate partial correlation between diffusion metrics and onset days (Table 2-11) when controlling for age in right thalamus μFA, and right amygdala μFA and a positive correlation in left caudate K_{lin} mean values. It should be noted that all the reported cognitive and imaging findings remained significant after excluding the six patients that required hospitalization at the onset of their illness.

Table 2-11: Partial correlation between Diffusion Metrics and Onset Days

Subcortical Structures	Days between onset of symptoms and MRI	
	***p*-Value**	***R*-Value**
L-Caud-K_{lin}	0.006*	0.423
R-Thal-μFA	<0.001*	-0.522
R-Amyg-μFA	0.007*	-0.418

* $p<0.01$, age was used as a covariate

2.4 Discussion

The long-term neuropsychological and cognitive effects of COVID-19 illness are still poorly understood. In the current study we examined the impact of COVID-19 illness on cognitive function approximately 8 weeks after infection and used 7T MRI to measure tissue microbleeds and changes in water diffusion metrics within subcortical brain structures of the limbic system associated with specific symptoms. The study cohort included people with predominantly mild COVID illness who reported neurological symptoms at the time of acute illness. They had measurable attention deficits compared to a normative population on average 53 days after the initial respiratory symptoms of COVID-19. The most prevalent radiological finding observed in 22 of 43 participants was white matter hyperintensities observed on FLAIR, consistent with those frequently attributed to small vessel disease and migraine. Evidence of abnormal susceptibility in GRE images indicative of microbleed was observed in four of 43 participants. Several changes in diffusion MRI metrics were observed within subcortical structures when grouping participants according to the presence and absence of specific symptoms. The presence of chills was associated with changes in MD in the thalamus. Diarrhea (GI symptom) was associated with diffusion metric changes in the caudate and putamen. Finally, the presence of nasal congestion and cough (respiratory symptoms) was associated with diffusion metric changes in the pallidum, accumbens, and amygdala. There was a weak correlation between attention scores and MD in the hippocampus. All cognitive and imaging findings remained significant after excluding the six participants that required hospitalization at the onset of their illness.

The deficit in attention observed on average 53 days from the onset of symptoms in this study cohort is consistent with previous studies on COVID survivors that have shown attention and concentration dysfunction between 10-35 days after hospital discharge [83], 85 days from recovery [163] and 60 days from recovery [164]. The time interval between the onset of acute symptoms did not correlate with cognitive performance. The most severe dysfunction observed in the current study were somatic symptoms (31.4%), PTSD (11.8%), and anxiety (9.8%). In participants experiencing PTSD, depression, anxiety, anger, and acute stress disorder scores indicated worse symptoms compared to the group without PTSD. Also, patients having decreased mood during their illness were at higher risk of depression compared to the group without decreased mood. PTSD, anxiety, and depression were previously reported to be significantly higher in COVID-19 survivors [11], [83]. Two participants had severe cognitive impairment, six participants had mild or moderate cognitive impairment, and 35 participants did not have any cognitive impairment (Table 2-6). Since the two participants with severe cognitive impairment were young (<40 years old) and there were no overall changes in cognition (RBANS total scale), or association between the length of time between symptom onset and cognitive testing, the impaired cognition detected in these participants may be attributable to the infection.

The most common neurological symptoms during the acute phase of the illness were brain fog (93%), headache (93%), change/loss of taste or smell (69%), fever (62%), and confusion (44%) and the most frequent non-neurological symptoms included fatigue (93%), anxiety (62%), PTSD (56%), decreased mood (49%), and acute stress disorder (51%). These findings are consistent with previous reports of fatigue, and problems with attention, anxiety, mood, and memory, as well as post-traumatic stress disorder (PTSD) following COVID-19 illness [8,11-14,28].

Interestingly, patients with gastrointestinal symptoms (e.g., diarrhea, nausea, vomiting and abdominal pain) during their acute illness had worse performance in some cognitive domains in the current study. This effect could be explained by the link between the gastrointestinal tract and the brain through the established gut-brain axis [125]. COVID-19 neuro-invasion may occur through the gut-brain axis via the enteric nervous system,

which is regulated by the vagus and symptomatic nerves and by the presence of ACE2 receptors in the enterocytes of the small intestine and colon [125], [165]. Those with diarrhea performed worse in the RBANS language domain, and those with decreased appetite/oral intake performed worse in the RBANS visuospatial/constructional abilities and total score. These cognitive findings remained significant after excluding the six patients that required hospitalization during their acute illness.

Radiologic evaluation of the MR images suggested that this cohort of COVID-19 survivors had white matter hyperintensities bilaterally in cerebral WM that were consistent with SVID in older subjects or previous history of migraines or COVID-induced migraines in younger subjects. The SARS-CoV-2 virus can produce neuroinflammation [125], [126], which plays an important role in the pathophysiology of migraines [166]. In participants with frontal lobe WM hyperintensities only one reported a previous history of chronic migraines. Therefore, we hypothesize that COVID infection might induce migraine-like symptoms in younger patients consistent with the headaches that patients experienced during their acute illness (headache was present in 93% of participants in the current study). Intra-axial susceptibility suggestive of microvascular pathology, ischemic and macro hemorrhagic manifestations have been previously reported in severe COVID cases [167]. In the current cohort abnormalities on GRE images consistent with microbleeds were noted in four of 43 patients (9.3%), which is less frequent than previous studies. One explanation for this discrepancy is that the participants in the current study were more mildly affected compared to the more severely ill patients included in previous studies. Specifically, only one participant required ventilation in the current study.

The volume of sub-cortical structures did not differ when comparing groups of participants with and without specific symptoms. There was a moderate correlation between left and right caudate volume, left thalamus volume, and left and right putamen volume and age. Although not observed in the current study, previous studies have shown volume changes in subcortical structures associated with gastrointestinal symptoms associated with irritable bowel syndrome (IBS), one of the most widely diagnosed disorders of gut-brain interaction [168]. For example, in one study patients

with diarrhea-predominant IBS had enlarged thalamus and caudate nucleus volumes and altered hemispheric asymmetries of these two structures [169]. In other studies, patients with vomiting had significantly lower volume of the right caudate and patients with chills had significantly lower volume of the left hippocampus [169], [170].

Tissue microstructural integrity in subcortical structures was assessed using diffusion MR imaging metrics. All structures except the hippocampus showed some changes in diffusion metrics associated with either neuropsychological, gastrointestinal, or psychiatric symptoms. It is unknown whether these microstructural alterations are due to the direct effect of the virus (neuroinvasion) or caused by a systematic reaction [125]. It was previously hypothesized that CNS neuroinvasion by SARS-CoV-2 could occur across the BBB or the blood-cerebrospinal fluid barrier at the choroid plexus, or through nerve routes such as the olfactory nerve, trigeminal nerve, gut-brain axis, or vagus-nerve [125], [171]. Follow-up studies will be needed to assess the longer-term impact of these observed change. It is noteworthy that MD in the hippocampus was moderately correlated with attention and total RBANS scale, FA and K_{lin} in the thalamus were moderately correlated with language, and μFA in the nucleus accumbens was moderately correlated with immediate memory. These changes observed across several subcortical structures suggest that subtle changes in several regions are contributing to different cognitive functions (Table 2-8).

One of the changes in the brain that occurs with aging is an increased presence of iron that can be identified with DTI. A previous study showed that both diffusion anisotropy and mean diffusivity were higher in an older group compared to a younger group in the caudate and putamen, while the thalamus showed a minor effect of age on anisotropy or diffusivity [172]. The current study showed a negative moderate partial correlation between diffusion metrics and the number of days to the onset of symptoms when controlling for age in right thalamus μFA, and right amygdala μFA and a positive correlation with left caudate K_{lin} mean values. This result suggests that some diffusion metrics may be changing as a function of time following infection.

2.5 Strengths and Limitations

The participants in the current study, selected from the LUC3 clinic in London, Ontario, had a confirmed diagnosis of COVID-19 infection and the referral criteria for the clinic were very broad (i.e. anyone 40+, or 18-39 with a comorbid condition). As a result, the population seen in clinic were representative of the overall COVID population in Ontario/Canada. The 7T neurological MRI scans were read by an experienced neuroradiologist Dr. Michael Jurkiewicz and the neurocognitive assessments were performed by a single trained rater and monitored by an experienced psychometrist and neurologist. Although the RBANS was initially developed for the assessment of dementia, the use of this assessment has been validated for screening neurocognitive status in a younger population with mild cognitive impairment [114]. However, RBANS is a brief battery and does not cover all aspects of cognition [146]. The use of ultra-high field 7T MRI in this study allowed the acquisition of diffusion MRI data to detect microstructural changes in the brain tissue with higher resolution than is typically used on clinical systems. The study also incorporated recently developed μFA measurements to increase sensitivity. The greatest limitations of the current study are the absence of baseline MRI, relatively small sample size and the lack of control groups who were infected with a different influenza virus or a healthy group. Without such controls it is difficult to confirm that the observed white matter hyperintensity and susceptibility changes are due specifically to the SARS-CoV-2 infection. However, since the white matter lesions observed in the current study had a very high prevalence (>50%) and were observed in many young participants, it is likely that the SARS-CoV-2 infection contributed to their development. The clinical impact of these changes is currently unknown.

2.6 Conclusions

The extent of neuroinvasion or changes in the brain following COVID-19 infection in relation to long COVID-19 is poorly understood. In this study, we assess the microscopic tissue integrity of the brain in-vivo using advanced diffusion tensor imaging (DTI) methods following COVID-19 infection with ultra-high-field 7 Tesla MRI and the association of these changes in the brain with long-term cognitive and

neuropsychological dysfunction. Results showed that the group performed significantly worse in the attention domain compared to the normative population. White matter hyperintensities on FLAIR images were the dominant radiographic finding and suggests that further follow up to assess the impact on neurological and neuropsychological disorders later in life may be beneficial. Differences in advanced diffusion metrics within several subcortical structures when comparing groups with and without symptoms also suggest subtle tissue changes in these regions may contribute to cognitive dysfunction following infection.

Chapter 3

3 Objectives, Conclusion, and future direction

3.1 Objectives

The overall goal of this thesis was to determine the incidence of brain imaging abnormalities in COVID-19 survivors who experienced neurological symptoms and the association of these brain injuries with long-term cognitive and neuropsychological dysfunction. We performed an observational cohort study that examined patients after they recovered from COVID-19 illness using the highest magnetic field strength available in Canada for human brain MRI. Ultra-high field MRI increases sensitivity to measure cerebral microbleeds, cerebral vascular integrity, and brain microstructural abnormalities related to ischemic tissue damage.

We hypothesize that COVID-19 patients with neurological symptoms would have impaired cognitive function associated with the number of microbleeds in the brain, the presence of white matter hyperintensities, and tissue microstructural changes in subcortical brain regions. When initially conceived, the primary endpoint for this cohort study was focused on was the incidence of microbleeds and the secondary endpoints included assessments of diffusion abnormalities and white matter hyperintensities.

Understanding the impact of imaging changes in the brain on cognitive function could allow patients to be managed more effectively, increasing their quality of life, and relieving future impact on the healthcare system.

3.2 Conclusion

In this thesis we aimed to detect brain structural abnormalities including microbleeds and cerebrovascular changes in COVID-19 survivors after recovery and associate these brain abnormalities with long-term cognitive and neuropsychological dysfunction using ultra-high field 7 Tesla MRI. We assessed microscopic tissue integrity of the brain using advanced diffusion MRI (dMRI) methods.

To determine if cognition is impaired in our sample, we used the RBANS battery that includes normative data for the analysis. The results showed that the population performed significantly worse in attention domain compared to the normative data in RBANS (p=0.008). We also examined if there was any association between the onset day of acute symptom and day of performing cognitive assessments but there were no significant correlations observed. Our study showed that 81% of the cohort included showed minimal cognitive impairments, 14% were mildly/moderately cognitively impaired, and 5% were severely cognitively impaired. The most severe dysfunctions were somatic symptoms, PTSD, and anxiety. Similarly, somatic symptoms, PTSD, acute stress disorder and sleep disturbance caused mild/moderate dysfunction in the population. All these neuropsychological findings necessitate further follow up with COVID-19 survivors to prevent complications later in life and increase their quality of life.

The dominant MRI finding in this cohort were white matter hyperintensities on FLAIR images and some of the patients showed susceptibility changes on GRE. We grouped patients based on the presence of specific symptoms and our results demonstrated differences in advanced diffusion MRI metrics within subcortical and limbic system structures between the group with and without symptoms.

Based on our findings of both cognitive and imaging assessments we suggest that these subtle brain tissue changes may contribute to the cognitive dysfunction following the infection. However further follow-up is needed in larger studies to determine whether these changes resolve or place the brain at increased risk for neurodegenerative disease in the future.

3.3 Future direction

The greatest limitations of this study were the small sample size and the lack of control groups either infected with a different influenza virus or a healthy group. Future studies should include control groups to compare the imaging and cognitive findings with to confirm that the observed results are due to SARS-CoV-2 infection. Currently, there are several larger scale imaging studies being conducted in different parts of the world (e.g. England, Canada), that could also provide either relevant control groups, or a means to answer some of the questions raised by the current study. However, none of these studies are performed at ultra-high magnetic fields. The use of ultra-high magnetic fields does provide some advantages including greater image resolution and sensitivity to microbleeds. However, because of the limited number of such MRI scanners available worldwide, large scales studies using such technology is not possible at this time.

This thesis was focused on the quantitative analysis of diffusion metrics withing subcortical structures. However, there is additional data available that has not yet been examined. For example, the MRI sequences included angiography and chemical exchange saturation transfer (CEST) imaging that could provide additional insights into brain changes following SARS-CoV-2 infection. In addition, diffusion metrics within specific white matter tracts could also be examined and related to cognitive changes. These data may provide information that could be used to generate hypotheses that could be tested using the larger cohort datasets that are currently being acquired around the world.

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Appendices

Appendix A 2: Ethics Approval for Human NeuroCOVID study

Date: 5 August 2020

To: Dr. Robert Bartha

Project ID: 116351

Study Title: Long Term Neurologic Effects of COVID-19 Illness

Application Type: HSREB Initial Application

Review Type: Delegated

Full Board Reporting Date: 18/Aug/2020

Date Approval Issued: 05/Aug/2020

REB Approval Expiry Date: 05/Aug/2021

Dear Dr. Robert Bartha

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
APA_DSM5_Level-1-Measure-Adult	Paper Survey	Received August 1, 2020	
APA_DSM5_Level-2-Anger-Adult	Paper Survey	Received August 1, 2020	
APA_DSM5_Level-2-Anxiety-Adult	Paper Survey	Received August 1, 2020	
APA_DSM5_Level-2-Depression-Adult	Paper Survey	Received August 1, 2020	
APA_DSM5_Level-2-Sleep-Disturbance-Adult	Paper Survey	Received August 1, 2020	
APA_DSM5_Level-2-Somatic-Symptom-Adult	Paper Survey	Received August 1, 2020	
APA_DSM5_Severity-of-Acute-Stress-Symptoms-Adult	Paper Survey	Received August 1, 2020	
APA_DSM5_Severity-of-Posttraumatic-Stress-Symptoms-Adult	Paper Survey	Received August 1, 2020	
Telephone_Interview_Cognitive_Status	Paper Survey	Received August 1, 2020	
Telephone_MoCA-Test-BLIND - 04-2020	Paper Survey		7.1
COLUMBIA-SUICIDE SEVERITY RATING SCALE-SSRS-Baseline_(UK-English) 1.14.09	Paper Survey	14/Jan/2009	
MRI_PatientScreeningForm_2017_final1	Paper Survey	07/Jan/2020	2.1
RBANS	Paper Survey	Received August 1, 2020	
LOI_NeuroCOVID3	Written Consent/Assent	01/Aug/2020	3
Neurological_COVID_7T_MRI_Study_Protocol V4	Protocol	01/Aug/2020	4

Documents Acknowledged:

Document Name	Document Type	Document Date
Updated Study Budget	Study budget	Received August 1, 2020

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB , except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Karen Gopaul, Ethics Officer on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Curriculum Vitae

Name: Helma Heidari

Post-secondary Education and Degrees:

Diagnostic Radiology Technology, Shiraz University
Shiraz, Iran
2008-2012 B.Sc.

Health System Management, Fanshawe College
London, Ontario, Canada
2019 Post-Graduate Certificate

Medical Biophysics, The University of Western Ontario
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2020-2022 M.Sc.

Scholarships and Awards: Western Graduate Research Scholarship (WGRS
2020-2022

Related Work Experience

Research Assistant
Robarts Research Institute**,** The University of Western Ontario
2020-2022

Presentations and Posters:

Helma Heidari et al., Long-term Effects of COVID-19 Illness in The Brain Assessed by 7T MRI
Imaging Network of Ontario 2021 Symposium
Toronto, Canada, Oral presentation
March 2021

Helma Heidari et al., Neurological and Neuropsychological Effects of COVID-19 infection in the Brain
London Health Research Day Event
London, Canada, Oral Presentation
May 2021

Helma Heidari et al., Long-term Cognitive and Neurological Effects of COVID-19 Illness Detected by 7T MRI
International Society of Magnetic Resonance in Medicine Annual General Meeting
Vancouver, Canada, Poster
June 2021

Helma Heidari et al., 7 Tesla Diffusion MRI in Subcortical Structures following COVID-19 infection
Imaging Network of Ontario 2022 Symposium
London, Canada, Poster
March 2022