CEREBRAL VASOMOTOR REACTIVITY TO ASSESS BRAIN DYSREGULATION IN POST COVID NEUROLOGICAL SYNDROME

by

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Dedication

I dedicate this thesis to my grandparents, Syed Rahim Uddin and Batool Sultana, who I lost during the time of me working on my statistics for this project. They raised me since the age of six and I live by the values instilled in me by them. You both will be missed. I want to express my sincere gratitude to my mother, Asra Mahmood, who has always been the core of my strength. My family, who is the source of my inspiration. A special thanks to my friends who never let me quit and who are the basis of my tenacity to push forward in life.

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Abstract

CEREBRAL VASOMOTOR REACTIVITY TO ASSESS BRAIN DYSREGULATION IN POST COVID NEUROLOGICAL SYNDROME

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Background: COVID-19 has wide-ranging physiological effects, with many patients complaining of persistent asthenia following recovery from the acute phase of the infection. The frequent term for this is Long Haul COVID (LHC). While we have tools to measure effects on general physiology in human subjects, a metric for cerebral dysregulation is lacking. Cerebral blood flow (CBF) is closely regulated in the healthy young person. Dysregulation has been well described in many conditions, including Posterior Reversible Encephalopathy Syndrome (PRES), and is associated with neurological deficits. Cerebral Vasomotor Reactivity was used as a tool to assess this dysregulation.

Methods: Transcranial Doppler (TCD) study for CVR was performed under the influence of Carbogen gas. A questionnaire collected prior to the procedure provided additional details on subjects demographics and COVID history. Cases and controls were recruited using self-reported questionnaire. Statistics involved assessing the reproducibility of the test as well as discovering differences between cases and control groups.

Results: CVR was assessed for 26 subjects. CBF velocity in the left MCA was analyzed at baseline, at peak Carbogen exposure, and in hypercapnic phase. The reproducibility of the test

was established within the longitudinal repeated measures data. The cases and control groups were insignificant in difference at base level but significant when controlled for confounders. CVR was found to increase by 3.76 units in cases compared to controls. Confounders like BMI, gender and age was found significantly different between cases and controls. Number of COVID episodes and symptom severity was significant for CVR.

Conclusion: This simple bedside test was found to be to be effective in producing a reactivity among all the subjects and was homogenous in its effect irrespective of baseline subject differences. As a preliminary test, the test showed differences among cases and control groups. The sample for the test lacked sufficient power and observations. A bigger sample size and a subsequent longitudinal follow up may help better understand the use of CVR to screen high-risk population for cerebrovascular anomalies.

Keywords

long-haul COVID (LHC), cerebrovascular reactivity (CVR), transcranial Doppler (TCD), carbogen, Cerebrovascular anomaly (CVA), cerebral blood flow (CBF), middle cerebral artery (MCA)

Chapter 1

INTRODUCTION

Background

COVID-19 has been declared the fifth pandemic of the century on March 11, 2020 and is currently affecting 213 countries, and is thus touted as the biggest global challenge since World War II. It is recognized as one of the biggest pandemics ever, when adjusted for time and population, and in terms of complications and mortality even though its mortality rate is heavily skewed towards advanced age. Its rapid infectivity and global impact led to it surpassing previous pandemics in numbers (post-adjustment). The pandemic has had an impact in all aspects of life including environmental, economic, social, political, and cultural (Bernal-Silva & Comas-Garcia, 2022; El-Shabasy, Nayel, Taher, Abdelmonem, & Shoueir, 2022; Graichen, 2021). The World Health Organization (WHO) estimates approximately 370 million confirmed global cases and close to 5.6 million deaths due to the virus by the end January 2022. The Center for Disease and Control and Prevention (CDC) estimates 74 million confirmed cases and a million deaths since 2020, in the United States by January, 2020.

COVID-19 belongs to the viral realm of Riboviria according to the International Committee on Taxonomy of Viruses (ICTV), a virus which uses homologous RNA-dependent polymerase for replication. Its family, Coronaviridae, is defined by an RNA virus which is enveloped, single stranded, genomes ranging from 25 to 32 kb (largest RNA genome in a virus), and a large spherical shaped spike protein 118–140 nm in diameter (giving its distinct crown shape, and thus the name), and a helical nucleocapsid. The family includes 39 species in 27 subgenera, five genera and two subfamilies (Letovirinae and Orthocoronavirinae). Phylogenetic classification identifies it to genus Betacoronavirus and the species “severe acute respiratory

syndrome-related coronavirus" (SARS) (Gorbalenya et al., 2020; Helmy et al., 2020; of the International, 2020; Payne, 2017; Randhawa et al., 2020). Since its recognition and addition into viral taxonomy, three distinct pandemic waves have been observed, and various new strains have been observed. Most of these variations are observed in its S or spike-protein and NSP3, and an estimated 26,844 single mutations were tracked in 203,346 human coronavirus 2019 genomes. These mutations and variations further curtail the efforts of curbing the infectivity of the virus and thus prompted special attention as "variants of concern" (Anastassopoulou, Manoussopoulos, Lampropoulou, & Tsakris, 2021; Graichen, 2021; Jia & Gong, 2021).

The clinical presentation of COVID-19 varied greatly. Asymptomatic individuals with a positive COVID test accounted for 20-75% in different demographic studies during peak pandemic era. COVID clinical presentation was further classified into mild, moderate and severe. Initial symptoms of pneumonia/mild pneumonia, diarrhea, cough and fever were classified as mild. Dyspnea, reduced oxygen saturation was classified as moderate. Respiratory failure, sepsis, organ failure was classified as severe. (Chen et al., 2020; Wu & McGoogan, 2020; Yanes-Lane et al., 2020).

The National Institute of Health (NIH) and CDC attribute any sequelae post-acute COVID-19 infection (four weeks post-infection), as Long COVID (Crook, Raza, Nowell, Young, & Edison, 2021). Respiratory and neurological signs and symptoms are the most common among the follow-up adults post two months. (Carvalho-Schneider et al., 2021; Couzin-Frankel, 2020; Garg, Arora, Kumar, & Wig, 2021; Halpin et al., 2021; Iqbal et al., 2021; Pergolizzi, LeQuang, Magnusson, Myrcik, & Varrassi, 2021; Yelin, Margalit, Yahav, Runold, & Bruchfeld, 2021). Several systematic reviews and meta-analyses have estimated approximately 15% -85% of the cases as having long-term neurological signs and symptoms (Anaya et al.,

2021; Collantes, Espiritu, Sy, Anlacan, & Jamora, 2021; Greenhalgh, Knight, Buxton, & Husain, 2020; Pavli, Theodoridou, & Maltezou, 2021; Scordo, Richmond, & Munro, 2021; Taherifard & Taherifard, 2020). Studies also suggest of neurological disorders and neurodegeneration due to COVID-19 infection (Ferini-Strambi & Salsone, 2021; McAlpine, Fesharaki-Zadeh, & Spudich, 2021). This led to a newer term, "post-COVID-19 Neurological Syndrome" (PCNS) (Camargo-Martínez et al., 2021; González-Herazo, Silva-Muñoz, Guevara-Martínez, & Lozada-Martinez, 2021; Nuzzo, Vasto, et al., 2021; Wijeratne & Crewther, 2021). The most common neurological complications were cerebrovascular disorders i.e., stroke, cerebral hemorrhage, and cerebrovascular thrombosis. Mild to moderate hypoxic injuries, infarcts, and microbleeds are the most common findings in brain autopsies of COVID-19 patients furthering the argument of COVID-19 cerebrovascular effects on the brain (Collantes et al., 2021; Fabbri et al., 2020; Jaunmuktane et al., 2020; Mukerji & Solomon, 2021; Reichard et al., 2020).

There is a growing concern with young healthy individuals with no significant clinical history, exhibiting marked cerebrovascular issues (strokes and hemorrhages) post COVID-19 infection (Oxley et al., 2020; Sashindranath & Nandurkar, 2021; Yaghi et al., 2020). Recent studies show a shift in the average age for earlier presentation of cerebrovascular events among general population as compared to pre-pandemic time (Katsanos et al., 2021; Siow et al., 2020; Yamakawa, Kuno, Mikami, Takagi, & Gronseth, 2020). Large vessel strokes and strokes in general, had an earlier mean age of 63 years (post-COVID) versus 74 years (standard), and 63 versus 70 years respectively. Cerebrovascular complications seem to be occurring as early as the 3rd and 4th decade of life among COVID survivors (Fifi & Mocco, 2020). Nannoni, et al. conducted a pooled meta-analysis of more than 100,000 individuals comparing COVID versus Non-COVID stroke presentation (Nannoni, 2021). They found patients with COVID-19 and

stroke were younger and had a higher National Institutes of Health Stroke Scale (NIHSS) measures compared to control population. These earlier shifts advocate a need of early, inexpensive screening or baseline diagnostic procedures among young individuals to help identify high-risk individuals among Long COVID survivors.

Cerebral Vasomotor Reactivity (CVR) is an indirect parameter for assessment of functionality of cerebral vasculature. It involves the use of external stimulatory technique to vasodilate and subsequently evaluate cerebral vessel dilation and resistance parameters through blood velocity recordings or imaging/mapping techniques. CVR deficit has been well established in research settings as an indicator of increased risk for stroke, cortical thinning, cognitive decline, mild cognitive impairment/dementia, carotid stenosis, neuropsychiatric disorders, neurodegenerative disorders, and overall mortality (Derdeyn, Grubb, & Powers, 1999; Gupta, Chazen, & Hartman, 2013; Miller, Howery, Harvey, Eldridge, & Barnes, 2018; Portegies, de Bruijn, Hofman, Koudstaal, & Ikram, 2014; Regan, Fisher, & Duffin, 2014; Rundek, Demarin, & Kittner, 1993; Sasoh et al., 2003; Silvestrini et al., 2000; Smeeing, Hendrikse, Petersen, Donahue, & Jill, 2016; Smoliński & Członkowska, 2016; Viticchi et al., 2012; Yonas, Smith, Durham, Pentheny, & Johnson, 1993).

The Transcranial Doppler (TCD) machine is commonly used to record cerebral blood flow recordings to calculate CVR. It is synonymous with "stethoscope for the brain" and is simple, non-invasive, non-radioactive, and inexpensive. It is very sensitive to blood flow changes and offers portable out-patient procedural technique to calculate CVR (Aaslid & Lindegaard, 1986; Alexander, Hennigan, Harrison, & Plotkin, 2021; Dahl et al., 1992; Fedriga & Czosnyka, 2021; Fisher & Mikulis, 2021; Gur, Bova, & Bornstein, 1996; Hugh Markus & Cullinane, 2001; HS Markus & Harrison, 1992; Matteis, Troisi, Monaldo, Caltagirone, & Silvestrini, 1998;

Ringelstein, Sievers, Ecker, Schneider, & Otis, 1988; Robba, Cardim, Sekhon, Budohoski, & Czosnyka, 2018; Webster et al., 1995).

There are various techniques to elicit a CVR response. Breath-holding, Acetazolamide injection and CO_2 inhalation are the routinely used external stimuli to prompt a CVR response. The current study uses Carbogen (95% O_2, 5% CO_2) inhalation as its external stimulus. The choice of Carbogen as the exogenous stimuli relies on it making the test universal for any demographic. It brings universality and standardization for the diagnostic procedure (Asghar, Hansen, Pedersen, Larsson, & Ashina, 2011; Mancino, Varesi, Cerulli, Aiello, & Nucci, 2011; Totaro, Barattelli, Quaresima, Carolei, & Ferrari, 1998). Carbogen helps remove the bias of hypoxia (associated with breath holding, tending to exaggerate vasodilatory responses/CVR measures) and increases the factor of reproducibility (McDonnell et al., 2013). It is a stimulus that can be stopped at any moment, if needed or lengthened if desired, without leading to a significant adverse event as compared to a dose of Acetazolamide injection which cannot be controlled after its application until its effects end (Fierstra et al., 2013; P. Liu, Jill, & Lu, 2019; Spano et al., 2013).

Study Aim: To assess if there is any significant difference in CVR between Post COVID Neurological Syndrome (PCNS) and non-symptomatic individuals.

Objectives/Specific Aims/Hypotheses:

a. To assess any demographic variables association with CVR among Post Covid Neurological Syndrome (PCNS) individuals against non-symptomatic individuals.

b. To access the efficacy/reliability of TCD and Carbogen as a bedside test among PCNS individuals.

Chapter 2

LITERATURE REVIEW

COVID-19

Since the first reported COVID-19 case on December 12, 2019, in Wuhan, China, the most populated city of central China, the phenomenal spread of the COVID-19 virus led to it being declared as the fifth pandemic of the decade, by the WHO on March 11, 2020, and affecting 213 countries and international travel and trade globally (Agrahari et al., 2021; Bhadoria, Gupta, & Agarwal, 2021). It started off as an outbreak of atypical pneumonia. The COVID-19 pandemic spread swiftly around the world, with initial epidemic numbers doubling every estimated 6.4 days. The agent responsible for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has the biggest viral genome, the capacity to infect birds, animals, and humans alike, and an estimated basic reproductive number (R naught) between 1.4 to 6.7. (Akbari et al., 2021; Asfahan et al., 2020; Bernal-Silva & Comas-Garcia, 2022; Liu, Gayle, Wilder-Smith, & Rocklöv, 2020; Sharma & Kumar, 2021; Swerdlow & Finelli, 2020; Wu, Leung, & Leung, 2020; Zhao et al., 2020). The COVID-19 pandemic statistics are rapidly evolving and ongoing since its declaration.

WHO estimates approximately 370 million confirmed global cases of COVID-19 and close to 5.6 million deaths by the end of January 2022. It brought an unprecedented global change in every interaction of life including the health, social, economic, and environmental sectors. Several inherent ambiguous factors in each country in terms of demographics, baseline national health, and public health responses and resources led to varied outcomes. The data is also clouded by the capacity of reporting, research, and release of data by countries. This ambiguous nature of pandemic spread, impact, and knowledge, further augments people's

confusion (Assefa et al., 2022; Tazerji et al., 2022). According to statistics, 50% of the global burden is shared between the North America and Europe (Happi & Nkengasong, 2022; Soneji, Beltrán-Sánchez, Yang, & Mann, 2021). Asia suffered disproportionate spread and impact among its countries and in fact was able to suppress the initial wave spike of the virus in heavily populated Southeast-Asian countries. (Amul, Ang, Kraybill, Ong, & Yoong, 2022; Hasib & Sekercioglu, 2022; Liang & Chen, 2022; Miyawaki & Tsugawa, 2022; Tang et al., 2022; Tazerji et al., 2022).

The case fatality rate (CFR) helps distinguish the severity due to COVID-19. It is estimated to be between 0% to 55%, rising with age. The CFR has the denominator of "Number of cases" which increased exponentially with increase in testing and testing capacity, thereby decreasing the CFR number overall in more industrial countries (Abdollahi, Champredon, Langley, Galvani, & Moghadas, 2020; Akbari et al., 2021; Gianicolo, Riccetti, Blettner, & Karch, 2020; Liang & Chen, 2022; Petersen et al., 2020; Tazerji et al., 2022; Vanella et al., 2022). The CFR in the COVID-19 first wave for United States was less than 1% for age group 20–54 years, 1–5% for age group 55–64 years, 3–11% for age group 65–84 years, and 10–27% in people aged 85 years and older (COVID et al., 2020).

The CDC estimates 74 million confirmed cases in the United States with close to a million deaths within the same period (CDC, 2022). The control of COVID-19 within United States was and continues to be vague and confusing, implying differences in policy making and public health mandates among the states (Lyu & Wehby, 2020; Xu et al., 2020). Reporting of maximum cases was in Northeast, South (sun-belt) and in the North (Great Plains zone) during the first, second and third wave, respectively (Vahabi, Salehi, Duarte, Mollalo, & Michailidis, 2021). There is substantial evidence documenting disparities in incidence and mortality based on

race, gender, co-morbidities, socioeconomic status, density, access to health and urban versus rural demographics (Clouston, Natale, & Link, 2021; Cuadros, Branscum, Mukandavire, Miller, & MacKinnon, 2021; Little et al., 2021; Lu et al., 2021; Matthews et al., 2021; Seligman, Ferranna, & Bloom, 2021; Zelner et al., 2021).

The Texas Department of State Health Services (DSHS) registered approximately 5 million cases in Texas in the past two years up to January, 2022. It puts Texas third, on the list of highest COVID-19 incidence states in the country, just behind New York and California (CDC, 2022). Harris County, around Houston, has the highest recorded cases with 950,282 cases by mid-February, 2022, followed by Dallas County at 482,298. Smith County recorded 28,671 cases within a population of approximately 200,000 which sets the crude attack rate for the population at approximately 15%.

Long COVID or Post-acute Sequelae of COVID-19 (PASC):

While most people are asymptomatic or present with subclinical symptoms, the infection gave birth to the field of Long COVID or Post-acute Sequelae of COVID-19 (PASC). The diagnosis is not yet specifically defined. The National Institute for Health and Care Excellence (NICE) define Long COVID as chronic or persistent symptoms without further explanation (Sykes et al., 2021; Venkatesan, 2021). The National Institute of Health (NIH) and CDC attribute any sequelae post-acute COVID-19 infection (four weeks after the infection) as Long COVID (Crook et al., 2021). The timeline of Long COVID or PASC varies, with some medical experts regarding 28 days as acute, while others extend it to 12 weeks and anything later than that as chronic. Currently, there are various definitions and criteria being proposed and summarized for Long COVID. Yong, et al, compiled a table of such definitions and timelines regarding Long-COVID. The Collaborative effort of NICE, the Scottish Intercollegiate Guidelines Network

(SIGN), and the Royal College of General Practitioners (RCGP) came up with a standardized guideline in differentiating Long COVID. Less than 4 weeks of symptoms is “Acute COVID”, 4-6 weeks of COVID symptoms is “ongoing symptomatic COVID-19 infection” and any signs and symptoms post 12 weeks of infection without underlying explanation is “Post-COVID 19 syndrome or Long COVID” (Fernández-de-Las-Peñas, Palacios-Ceña, Gómez-Mayordomo, Cuadrado, & Florencio, 2021; Leen, 2021; Shah, Hillman, Playford, & Hishmeh, 2021; Yong, 2021).

Long COVID/PCNS Epidemiology:

Though coined in an arbitrary fashion, a Long-COVID classification is necessary today as a guideline for a prospective rise in complications and sequelae (Alwan, 2021; Gorna et al., 2021). Several studies about Long-COVID indicate fluctuating/relapsing symptoms, severe organ damage, and rise in multisystem complications, irrespective of age or severity of preliminary COVID infection. Approximately 55 varied, lingering, long-term effects have been recognized in various organ systems due to Long COVID (Lopez-Leon et al., 2021). Signs and symptoms persistence in COVID-19 cases is seen in up to 20-87% of affected individuals, with age, gender, organ system, severity, and hospitalization as the key differentiating factors for the residual effect (Iqbal et al., 2021; Pavli et al., 2021). The typical timeline for Long COVID is two to three months of signs and symptoms. Persistence of at least one post-acute sequalae in the first month, at 2-5 months and at 6 or more months were 54% (45–69%), 55% (34.8–65.5%), and 54% (43.5–67.0%) respectively (Groff et al., 2021). The research, though, is suggestive of a need of comprehensive studying of COVID-19 survivors for a year or two post-infection, and some evidence suggesting up to 7 years. Respiratory and neurological signs and symptoms are the most common symptoms post two months, with two-thirds of the cases reporting at least one of

these, even with sub-clinical acute COVID history (Carvalho-Schneider et al., 2021; Couzin-Frankel, 2020; Garg et al., 2021; Halpin et al., 2021; Iqbal et al., 2021; Pergolizzi et al., 2021; Yelin et al., 2021).

Long COVID Neurological Sequelae or Post-COVID-19 Neurological Syndrome (PCNS):

Studies show that as high as 53% of post-mortem cases of COVID-19 had viral mRNA or proteins found in their brains (Matschke et al., 2020). Several systematic reviews have speculated approximately 40% -85% of the cases as having specific or non-specific neurological symptoms with COVID-19 (Anaya et al., 2021; Collantes et al., 2021; Scordo et al., 2021; Taherifard & Taherifard, 2020). These specific and non-specific signs and symptoms range from acute phase early anosmia and headaches to long-term brain fog, fatigue, memory loss, lethargy, myopathy, toxic/metabolic encephalopathy, stroke, seizures, and hypoxic/ischemic brain injury (Kihira et al., 2021; Nuzzo, Cambula, et al., 2021; Pinzon, Wijaya, Buana, Al Jody, & Nunsio, 2020). Neurological symptoms are some of the earliest presentations in acute settings occurring as early as two days after COVID infection. Neurological events within hospitalized patients were found to be an independent predictor for increased mortality when controlled for other factors (Beghi et al., 2021; Kim et al., 2021; Salahuddin et al., 2020). All of these factors led neurologists to alienate neurological sequelae as post-COVID-19 Neurological Syndrome (PCNS)(Camargo-Martínez et al., 2021; González-Herazo et al., 2021; Nuzzo, Vasto, et al., 2021; Wijeratne & Crewther, 2021). Long-COVID, though new in the current context, was suggested long before the ongoing pandemic in relation to the previous SARS (2003) endemic. With the predecessor SARS-2003 strain, the most profound post-infection effects were found in the nervous system lasting up to 4-7 years.

Neurological sequelae are an expected phenomenon due to established neurotrophy or the affirmed hematogenous spread to the Central Nervous System (CNS) by COVID-19 virus (Proal & VanElzakker, 2021). The most common neurological complications generated within the current strain of coronavirus are cerebrovascular disorders, i.e., stroke, cerebral hemorrhage, or cerebrovascular thrombosis. Mild to moderate hypoxic injuries, infarcts, and microbleeds are the most common findings in brain autopsies of COVID-19 patients, furthering the argument of COVID-19 cerebrovascular effects on the brain (Collantes et al., 2021; Fabbri et al., 2020; Jaunmuktane et al., 2020; Mukerji & Solomon, 2021; Reichard et al., 2020).

The Neurotrophic and Cerebrovascular Etiology of Coronavirus

The clinical and experimental data show several means of etiology for COVID-19 neurotrophic and cerebrovascular events. One primary theory is the cytokine storm of viral etiology. Increased pro-inflammatory cytokines interleukin (IL) and T cell response towards the viral infection and its subsequent effect on vascular endothelium is deemed primarily responsible for cerebrovascular pathology. To support this theory, several macro and micro hypoxic/ischemic injuries and infarcts have been detected in COVID-19 death autopsies. Decreased glutathione, upregulation of inflammation- and immune-related genes IL1B, IL6, IFITM, MX1, and OAS2, and neuroinflammation markings in autopsies have been detected (Boroujeni et al., 2021; Kantonen et al., 2020).

Direct neurotrophic action and coagulative vasculopathy is another etiology for cerebrovascular anomalies seen in extreme cases of Disseminated Intravascular Coagulopathy (DIC) with thrombotic occlusions, hemorrhages, and angiopathy (Kihira et al., 2021). Immune-mediated damage is another etiology suggested with central and peripheral neuropathies. Several case studies show increased titers of antiganglioside antibodies such as serum anti-GD1b IgG for

Guillain-Barré syndrome (GBS), a sudden and significant rise in number of Peripheral Nervous System (PNS) disorders, and polyneuropathies with preceding COVID-19 infection, all indicating an immune mediated damage by the virus (Abu-Rumeileh, Abdelhak, Foschi, Tumani, & Otto, 2021; Civardi, Collini, Geda, & Geda, 2020; Guilmot et al., 2021; Trentinaglia et al., 2022; H. Zhao, Shen, Zhou, Liu, & Chen, 2020).

By far, the most prominent etiology assumed is the expression of various human receptors for the COVID-19 virus in the brain. Human Angiotensin-converting enzyme 2 (ACE2, the attaching receptor for the SARS virus) is one of the most recognized and prominent receptors found in the brain in microbiological studies. Several other receptors such as the serine activated transmembrane protease serine 2 (TMPRSS2), or activate neuropilin (NRP1 and NRP2) receptors, CD 147, and many more receptors are hypothesized as other receptors found in the brain which could possibly explain COVID-19 neural tropism. Observations of angiocentric mixed inflammatory infiltrate, increased vascular permeability, and microvascular microthrombi lend support to this later etiology. In vitro demonstration of vascular damage and endothelins on blood vessel organoids furthers the ACE-2 receptors cerebrovascular damage theory (Amezcua et al., 2020; Cantuti-Castelvetri et al., 2020; Fodoulian et al., 2020; Qiao et al., 2020). Most of the above etiologies (cytokine, direct, immune-mediated or receptor trophic) are interlinked, and illness could possibly be an interplay of all or some of the etiology mechanisms, as a basis for these neurological/cerebrovascular findings.

Neurotrophic and Cerebrovascular/Cerebral Ischemic Pathology of Coronavirus

Human, mice, and organoid studies show extensive direct neurotropic viral invasion and high replication of the SARS virus within the neurons. Besides neural death found in these experiments, the metabolic hypoxic changes in adjacent infected neurons is a significant finding

in these studies (Soltani Zangbar, Gorji, & Ghadiri, 2021; Song et al., 2021). Increased evidence on mapping studies shows local hypoxic environments around these neurons and vascular disturbances. Microscopic and autopsy examinations in most patients showed acute hypoxic injury in the cerebrum and cerebellum, with loss of neurons in the cerebral cortex, hippocampus, and cerebellar Purkinje cell layer indicating a focal and global hypoxic impact on the overall brain structure (Paniz-Mondolfi et al., 2020; Solomon et al., 2020; Song et al., 2021; Thakur et al., 2021). Scientists hypothesize a series of pathobiological changes due to COVID-19 infection which imitates the pathology of an acute ischemic stroke inflammatory process. Transcription factors such as nuclear factor kappa B, NOD-like receptor protein 3 inflammasome via pattern recognition receptors, and damage-associated molecular patterns (DAMPs), are all pro-inflammatory indicators of bioenergetic failure found in COVID-19 patients similar to stroke pathology (Wijeratne, Gillard Crewther, Sales, & Karimi, 2021). These microscopic hypoxic findings together with inflammatory markers are indicative of local and globalized brain ischemic changes and possible immediate and future infarcts, higher stroke probability, delirium, and neurocognitive impairment. Thus, there is a need to augment research in neurology in these areas and open up more extensive studies for neurodegeneration and cerebrovascular studies and reduce future neurological burden (Aghagoli et al., 2021; Boldrini, Canoll, & Klein, 2021).

Cerebrovascular complications in young Long COVID individuals

There is a growing concern with young, healthy individuals with no clinical history exhibiting marked neurological issues post COVID-19 infection. Recent studies show a shift in the mean age for earlier presentation of neurological complications. These neurological issues are predominantly in the form of large vessel ischemic strokes and intracranial hemorrhages specifically in the younger population (Oxley et al., 2020; Sashindranath & Nandurkar, 2021;

Yaghi et al., 2020). Large vessel strokes had a mean age of 74 years compared to 63 years found in post-COVID patients. Stroke, in general, had a mean age of 70 years as against a mean of 63 years seen in post-COVID patients. Many neurological complications seem to be occurring as early as the 3rd and 4th decade of life among COVID-19 survivors. This is odd compared to what was happening previously in the pandemic (Fifi & Mocco, 2020). Nannoni, et al. examined a pooled meta-analysis of more than 100,000 individuals comparing COVID-19 versus non-COVID-19 stroke presentation (Nannoni, 2021). They found that patients with COVID-19 and stroke were six years younger and had higher National Institutes of Health Stroke Scale (NIHSS) measures compared to the non-COVID-19 control population. The greater odds of early incidence of various cerebrovascular events have been a recurring theme in many such research analyses (Katsanos et al., 2021; Siow et al., 2020; Yamakawa et al., 2020). These shifts in the mean age of neurovascular events raise the concern for growing neurological complications in the population in the near future.

The increased risk of stroke, cognitive disabilities, neuropsychiatric issues, and theories of increased risk of neurodegenerative disorders owing to the COVID-19, is being extensively studied. In an effort to establish long-term neural microvascular and cerebral blood flow due to COVID-19 changes, a recent study using MRI brain imaging was done. The results showed a significant decrease in cortical thickness and white matter microstructural changes in severely affected and mildly symptomatic patients with COVID-19 infection versus the healthy controls (Qin et al., 2021). Registries of people with COVID-19 are being formed in an effort to assess and combat such cerebrovascular findings. Incidentally, some research suggests starting anticoagulation therapy in positive individuals (Fifi & Mocco, 2020). These findings suggest a

need for early and inexpensive screening with baseline diagnostic procedures, among mid-aged individuals to help identify who is at high-risk for Long COVID.

Cerebral Vasomotor Reactivity as a measure for Global Cerebral Ischemia assessment

Cerebral ischemia entails a reduction in Cerebral Blood Flow (CBF) or Cerebral Perfusion which is maintained by Cerebral Autoregulation (CA) (rigid interplay between flow, pressure, perfusion, resistance homeostasis). CA can be affected by neurotropic infections (Elkind, Boehme, Smith, Meisel, & Buckwalter, 2020; Lassen, 1959; Paulson, Strandgaard, & Edvinsson, 1990; South et al., 2020). Global ischemia encompasses a generalized reduction in blood flow. Normal CBF values range between 50 to 75 mL/100 g of brain tissue per minute and varies in different areas of the brain. A decrease of approximately 18 mL/100 g of brain tissue per minute and 10 mL/100 g of brain tissue per minute is considered the basis for ischemic depolarization and subsequent ischemia or neuronal death, respectively (Bhardwaj, Alkayed, Kirsch, & Hurn, 2003; Harukuni & Bhardwaj, 2006). Even though decreased CBF is a parameter for ischemic damage, it is a snapshot at a given time and place rather than a more comprehensive longitudinal cerebral vasculature insult. CBF is heavily influenced by physiological, biochemical, and pathological parameters (i.e., cerebral perfusion, mean arterial pressure, neural activity, cerebral metabolism, and arterial CO_2).

CO_2 is one of the most potent modulators of cerebral blood flow and is unique to cerebrovasculature. It directly impacts blood vessel wall pH, decreasing cerebral flow resistance (increasing blood flow) and competes with perfusion pressure for vessel wall regulation. Flow velocity varies from a 3–6% increase to a 1–3% decrease per millimeter of mercury change in CO_2. Detailed implications of each of these factors and their role in blood flow regulation, with vasomotor reactivity, have been extensively studied, yet incompletely understood (Ainslie &

Duffin, 2009; Derdeyn et al., 1999; Fantini, Sassaroli, Tgavalekos, & Kornbluth, 2016; Kainerstorfer, Sassaroli, Tgavalekos, & Fantini, 2015; Lam, 2021; Markwalder, Grolimund, Seiler, Roth, & Aaslid, 1984; Regan et al., 2014; Christopher K Willie, Tzeng, Fisher, & Ainslie, 2014).

Adequate brain blood perfusion and blood supply is vital to support normal aging, and navigate acute and chronic illness (Fantini et al., 2016). Cerebral Vasomotor Reactivity (CVR) is an increasing phenomenon in CBF, in vivo, following an increase in CO_2, the effects being mediated at the cerebral arterioles level. When measured, it is the ratio of blood flow (a compensatory vascular dilation) to exogenous stimuli (CO_2, Acetazolamide, breath-holding, etcetera). Hypercapnia (i.e., increase in $PaCO_2$) increases CBF, whereas Hypocapnia (i.e., decrease in $PaCO_2$) decreases CBF (Ainslie & Duffin, 2009; Smoliński & Członkowska, 2016; C. Willie et al., 2011). The use of CVR relies on its ability to reflect cerebral vascular constriction and dilation capability and is widely regarded as a measure of cerebrovascular function.

One of the major uses of CVR is in its ability to assess reserve capacity of cerebral circulation in patients with cerebrovascular disease. It is a performance metric test, which reflects the responsiveness of particular regions' vascular resistance and perfusion pressures by assessing the CBF parameters (Duffin et al., 2018; Tomoto, Riley, Turner, Zhang, & Tarumi, 2020). In simple terms, it is a stress induced measure to check cerebral vasculature's capability of adapting to blood flow changes through its resistance parameters. The ability of cerebrovascular vessels dilation capacity for acute and chronic stress is limited. The underlying physiology for CVR is, if there is decrease in perfusion in a certain region in the brain, the parallel/collateral vasculature should help compensate for this loss by vasodilation and there should be enough

reserve to help substantiate the loss, commonly referred to as “steal” (visualized in MRI by Voxel change, TCD implied by flow change). Reduction in blood flow is thus synonymous to this vascular steal (Jorn Fierstra et al., 2010; Harper, 1966; Mandell et al., 2008; Symon, 1968; Tsivgoulis & Alexandrov, 2008).

Loss of vasomotor reserve indicates diminishing compensatory vasodilatation. This leads to vessels relatively more dilated as compared to its normal baseline. Thus, these vessels lose its intrinsic vasodilatory capacity to maintain sufficient blood supply and additional autoregulatory needs cannot be met during a subsequent ischemic insult., increasing susceptibility to brain ischemia and consequences of other perfusion deficits. Invoking this global vasostimulatory effect and assessing the underlying vasodilatory reserve is the foundation behind vasomotor reactivity testing and it being deemed as a biomarker for cerebrovascular health (Bhogal et al., 2014; Derdeyn et al., 1999; Duffin et al., 2018; Fisher & Mikulis, 2021; Ringelstein et al., 1988; Sobczyk et al., 2014; Sobczyk et al., 2021).

A decrease in CBF response compared to standard (post vasodilatory stimulation) is evidence of impaired hemodynamic status in an individual. It is an indirect means to show the microvasculature defects of the cerebrovascular tree and gives one a snapshot of the underlying pathology and cerebrovascular reserve function (Dahl et al., 1992). Over the years, there has been no standard procedural method for CVR measurements. Thus, addressing the limitations of CVR studies is a major topic of research among researchers globally for the past two decades. Where CVR is indirect blood flow measure, Pulsatility Index (PI) is another additional measure which helps one understand the Cerebral Perfusion Pressure (CPP) defects of cerebral autoregulation. It is inversely proportional to CPP, and it helps one overcome vessel diameter dilation bias, one of the biggest variable confounders for CVR assessment (i.e., the proximal

major vessel dilation during stimuli skewing the distal resistance CVR parameters). It adds validity to CVR by assessing distal cerebrovascular flow resistance. A low blood flow velocity, high PI and low CVR has been a traditionally more accepted, yet conflicting, parameter for cerebrovascular reserve loss (Shim et al., 2015). PI has been known to reflect cerebral perfusion pressure issues, though its results are often inconsistent, questioning its reliability (Bellner et al., 2004; de Riva et al., 2012; McQuire, Sutcliffe, & Coats, 1998). The Gosling Pulsatility Index is the most studied TCD waveform value and a common inbuilt calculated feature of most TCD machines. It is the difference between systolic and diastolic flow velocities divided by the mean velocity using peak-to-peak amplitude (PI = (peak systolic velocity - minimal diastolic velocity) / (mean velocity); pulse amplitude to its mean value) (Gosling & King, 1974).The relationship between CVR and PI is controversial but widely accepted (Czosnyka, Richards, Whitehouse, & Pickard, 1996; Michel & Zernikow, 1998).

Decreased CVR has been well established in research settings as an indicator of increased incidence of stroke, cortical thinning, cognitive decline, mild cognitive impairment/dementia, carotid stenosis, neuropsychiatric disorders, neurodegenerative disorders, and overall mortality (Derdeyn et al., 1999; Gupta et al., 2013; Miller et al., 2018; Portegies et al., 2014; Regan et al., 2014; Rundek et al., 1993; Sasoh et al., 2003; Silvestrini et al., 2000; Smeeing et al., 2016; Smoliński & Członkowska, 2016; Viticchi et al., 2012; Yonas et al., 1993). In theory, CVR helps assess hemodynamic impairment, blood reserves, and contralateral flow pathways. Current studies in high-risk asymptomatic individuals, such as the “Carotid Revascularization and Medical Management for Asymptomatic Carotid Stenosis Trial (CREST-II)”, the “Carotid Revascularization and Medical Management for Asymptomatic Carotid Stenosis–Hemodynamics trial (CREST-H)”, and the “The Asymptomatic Carotid Surgery Trial-2

(ACST-2)", this cohort can open up further doors and advances in neurological public health advances, neurological intensive care, and other neurodegenerative disorders research. Loss of cerebral hemodynamics form an essential intersection for most of these trials and applications (Fisher, Venkatraghavan, & Mikulis, 2018; Howard et al., 2017; Pelz, Lownie, Mayich, Pandey, & Sharma, 2021; Sobczyk et al., 2020).

Tools for measuring CVR (TCD and Carbogen)

Recognizing the high number of PCNS events, there is a call for neurological monitoring, irrespective of age, severity of illness, and symptomatology of post-COVID-19 survivors (Camargo-Martínez et al., 2021; Wijeratne & Crewther, 2020; Wijeratne et al., 2021; Yelin et al., 2021). The most common techniques in assessing the severity of cerebrovascular events currently in place for COVID-19 patients are CT-Scan, PET scan, MRI, or hematological assay. An effective, reliable, non-invasive, and non-radiological assay to assess underlying cerebrovascular effects is required. TCD is the so-called "stethoscope for the brain" and is a simple, non-invasive, non-radioactive, inexpensive out-patient procedural technique to calculate CVR, which is an indicator of global cerebral hemodynamics. TCD has been used for over four decades to assess CVR. The National Stroke Association organized a panel, in 1997, to assess the significance of TCD in its role for characterizing cerebrovascular issues and prevention (Alexandrov et al., 1998). They found that calculating cerebral blood flow measures and PI makes TCD a feasible bedside assessment tool in neuroimaging milieu (Aaslid & Lindegaard, 1986; Alexander et al., 2021; Dahl et al., 1992; Fedriga & Czosnyka, 2021; Fisher & Mikulis, 2021; Gur et al., 1996; Markus & Cullinane, 2001; HS Markus & Harrison, 1992; Matteis et al., 1998; Ringelstein et al., 1988; Robba et al., 2018; Webster et al., 1995).

The use of Inhaled Carbogen (95% O_2, 5% CO_2) as the exogenous stimulus for CVR makes it applicable for a wide patient population and can be standardized as a stimuli for the preliminary procedure. The safety and efficacy of inhaled CO_2 gas mapping of CVR have been studied in the past across all age groups. The other options, breath-holding is subjective to individuals' respiratory tidal volumes, physiological lung volume contraction factors, and inspirational volumes. Acetazolamide is invasive, known to cause adverse/long-lasting effects, and hard to control in the event of an adverse reaction during the procedure itself (Asghar et al., 2011; Mancino et al., 2011; Totaro et al., 1998). The feasibility of Carbogen relies on it being a stimulus that can be stopped at any moment or lengthened if desired, without leading to problems. It is universal, cost friendly, has minimal toxicity, and is homogenous with atmospheric air constituents and easily excreted. Studies have shown complete recovery without any neurological sequelae even after accidental iatrogenic hypercapnia, and it is safe even among critically ill patients (J1 Fierstra et al., 2013). In one study, transient events such as shortness of breath, headache, and dizziness were found in only 1 out of 10 individuals (only during the hypercapnic phase) with no neurological ischemic or major complications found later among clinical patients (Spano et al., 2013).

The use of Carbogen increases reliability, compared to other methods (Totaro, Marini, Baldassarre, & Carolei, 1999). CO_2 is one of the most sensitive stimuli and also helps reach the hypercapnic assessment goal much quicker (McDonnell et al., 2013). Carbogen delivers rapid onset, allows for easier maneuvering, has a strong potency for vasodilation (P. Liu et al., 2019). For this study, we use an open-circuit technique where individuals inhaled a fixed fractional concentration of 5% CO_2 (Carbogen 95% O_2, 5% CO_2). This technique is one of the most common, low in specification, relatively inexpensive and commonly used in clinical settings for

disease risk, health status and efficacy measures (Burley, Lucas, Whittaker, Mullinger, & Lucas, 2020; Portegies et al., 2014; SS Meel-van den Abeelen, Lagro, van Beek, & Claassen, 2014).

The choice of Middle Cerebral Artery (MCA) relies on it being the major terminal branch of internal carotid artery and thus its extensive supply of oxygen to the core brain, supplying almost 80% of the brain (COVID patients most affected zones) and its easy accessibility (skull is the thinnest in adults). Its blood flow parameters correlate well with hemispherical brain flow changes and Peebles et al., compiles prior research showing MCA velocity changes effectively reflecting global blood flow changes (Peebles et al., 2007; Shigemori et al., 1992). The MCA supplies a larger area of the brain without having a profound vasodilatory effect on itself with the exogenous stimuli (CO_2), controlling relatively for vasodilatory bias (Bishop, Powell, Rutt, & Browse, 1986; Jarrett et al., 2020; McDonnell et al., 2013; Shigemori et al., 1992).

The subsequent research proposed to assess the efficacy of Vasomotor Reactivity and Global cerebral vasculature dynamics to assess presence or absence of subclinical hemodynamic impairment in Long COVID patients. It is an effort to come up with a non-invasive, non-radiological, inexpensive, universal, preliminary out-patient neurological cerebrovascular assessment test for anyone with a history of COVID.

The next chapter outlines methods used in this study.

Chapter 3:
METHODOLOGY

Subjects and Setting:

Healthy adults between the ages of 18 and 40 years of age were eligible for enrolling on a volunteer basis in the research study. The inclusion criteria include self-reported Polymerase Chain Reaction (PCR) or an antibody test positive for COVID-19 three months prior to the test date for cases based on PCNS definition. The three-month lag time is to avoid misclassification due to ongoing acute inflammation or infection. Since cerebrovascular reserve loss itself is a sign, presence of it, is evidence for these subjects as being under PCNS. This age group helps assessing the underlying risk in the third and fourth decade of life among COVID-19 survivors, as recognized as high-risk cohort for assessment within the literature. Past or present history of respiratory or cardiac/vascular issues, neurological disorders, autoimmune disorders, or drugs which affect the vascular dynamics formed the exclusion criteria (see Appendix 1 for more details). Demographics and behavioral variables which can influence CVR like sex, age, smoking, and BMI were used as covariates rather than exclusion criteria to further the investigative approach of this study. Age and CVR was assessed as a continuous variable. Rest of the variables were categorized into categorical groups of yes and no. Acute or chronic symptoms experienced due to COVID were recorded for statistics (Appendix 2).

The research was conducted on the 9th floor, Clinical Research and Neurological Departments at UT Health, Plaza tower, Tyler, Texas. Dr. George Plotkin (MD, PhD) is the head of the Neurology Department. Southwestern Cerebral Circulatory Dynamics (SCCD) is the adjoining ultrasound service provided adjacent to the neurology department. SCCD was started in 1991 by Thomas Howard Alexander (BS, VT), with the mission of providing quality training for physicians, technologists, and nurses in cerebral hemodynamics. Their TCD is unavailable

anywhere else but Europe. SCCD has performed 28,000 transcranial Doppler studies ordered by neurologists in Tyler, Texas, providing the medical community with a clinical service normally not available in a town with a population of 100000 (Prior research accomplishments of the department are reported in Appendix 3).

Procedure:

Blood pressure and neurological assessment was conducted for all subjects prior to testing. Subjects were asked to refrain from food, smoking, coffee, and alcohol, or any involvement in heavy workouts four hours prior to testing. They were tested in sitting position for TCD (M. Y. Zhao et al., 2021). Subjects were briefed on any effects they may feel during the procedure, with a rundown of specific instructions on their role during the procedure. A signed informed consent was acquired prior to the start of procedure (Appendix 4). A preset 1.6- to 2.0-MHz pulsed- robotic TCD transducer system was used to insonate at 50-55 mm at the temporal window by an experienced blinded technician, just above the zygomatic arch (transtemporal window), in order to access the continuous flow in the MCA.

The TCD protocol involved marking the best window for TCD monitoring above the zygomatic arch on the left side and fitting the robotic headband to sit on that spot. The hydraulic robot driver is most helpful in capturing the general Left Middle Cerebral Artery (LMCA) waveform and then adjusting on the optimum waveform. Ultrasound gel was applied at the probe skin interface. A 2 MHz single crystal pulsed Doppler is the transmitter frequency of choice. Depths of insonation for the LMCA ranged from 50 to 60 mm with the pulsed gate set at 14-18 mm. Doppler gains and amplitudes were adjusted for waveform optimization. A Flexicare Dual Adult Mask allowed for gas delivery and CO_2 monitoring (Figure 2).

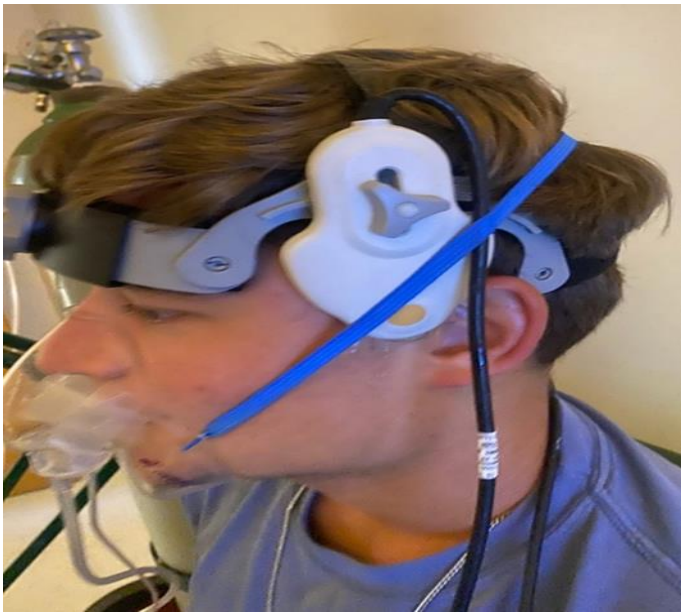


Figure 2. TCD hydraulic Monitoring headband in place with Dual-Port disposable face mask.

The gas used for provocation was a compressed gas mixture of 5.16% Carbon Dioxide with the balance being Oxygen (commonly called Carbogen). FDA approval was obtained for this application (Figure 3).

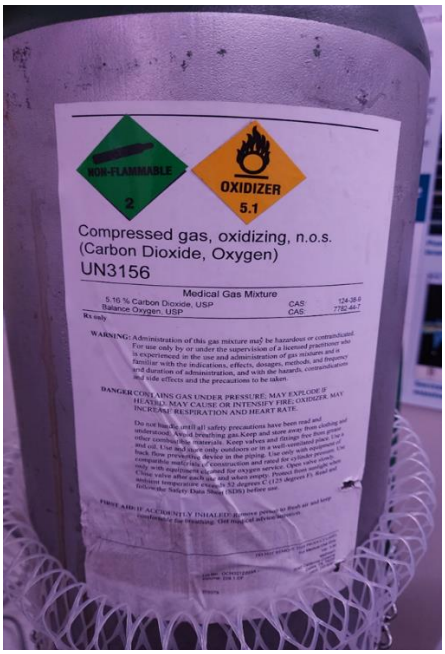


Figure 3: Carbogen Tank

A dual function regulator was used to step down the gas pressure from 1900 psi in the full tank to 15 psi during the inhalation part of the provocation protocol. A standard 3-minute challenge of iso-oxic hypercapnea gas (Meduna's mixture -95% O_2, 5% CO_2) was inhaled by the individuals followed by a minute of hyperventilation (hypocapnia) to elicit a full vasomotor response on maximal dilation and constriction. This challenge was divided into two similar stimuli separated by a small interval to induce familiarity, reduce any physiological reflexes, and to compare the reliability of results. A standard interval period of extended normal air breathing through the mask preceded and superseded the gas stimulus till steady measures were recorded. Dual mask help maintain record of Partial End Tidal Carbon Dioxide. Blood Pressure was recorded prior to the challenge and at the end of the test. All parameters were stored in real-time and reviewed off-line to insure accurate calculations. Institutional review board (IRB) approval was obtained for this research study.

Test Protocol

Test subjects were subjected to the following 22-minute protocol after the monitoring TCD probe was fixed to the left MCA and the disposable dual port mask was affixed. Mean flow velocities (MFV) were continuously recorded during each stage (Figure 1).

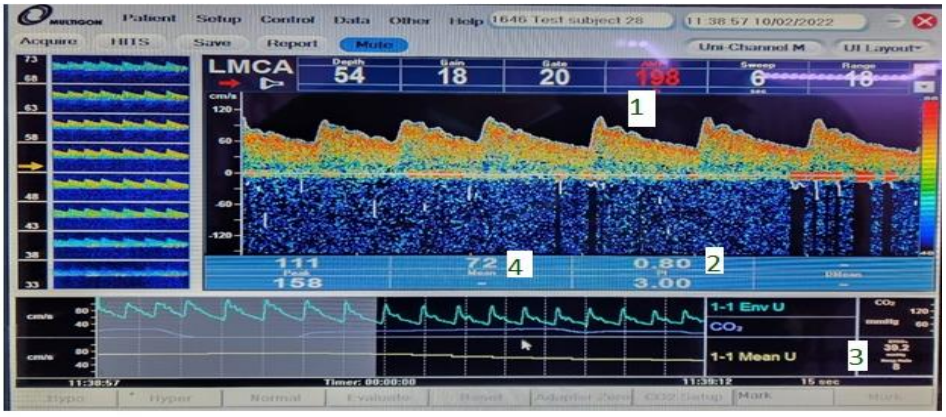


Figure 1: TCD Screen snapshot. 1. A real-time spectral display of the left middle cerebral artery with mean flow velocities (MFV) and Gosling pulsatility indices. 2. End-tidal CO_2. 3. Respiration rate. 4. Blood velocity at 10cm/sec

Stage 1 – minute 0-6 – normal breathing (Baseline Phase 1; B1)

Stage 2 – minute 7-10 – breathing carbogen gas at 15 psi (Stimulus phase 1; S1)

Stage 3 – minute 11 – hyperventilation with gas off (Hypocapnic phase 1; H1)

Stage 4 – minute 12-13 – normal breathing (Baseline phase 2; B2)

Stage 5 – minute 14-17 – breathing carbogen gas at 15 psi (Stimulus phase 2; S2)

Stage 6 – minute 18 – hyperventilation with gas off (Hypocapnic phase 2; H2)

Stage 7 – minute 19-22 – normal breathing (Baseline phase 3; B3)

The CVR is calculated by taking the maximum MFV (mean flow velocity) calculated during Stage 5 minus the minimum MFV calculated at the end of stage 6 divided by mean MFV calculated during Stage 1.

CVR= (MFV peak hypercapnia – MFV peak hypocapnia)/MFV peak baseline

Sample size was calculated using samplesizecalc.com. A sample size of 96 individuals were needed to show any significance at a probability of 95%, with the power set at 80%. Data analysis included two separate computations. 1. First for reliability and reproducibility of the procedure. Multilevel longitudinal repeated measures analysis was performed to assess this. 2. Data analysis for group differences. T-test analysis was performed to access CVR mean differences between both groups, followed by multivariable regression analysis to include confounders. Bivariate regression analysis was performed for Cases only parameters to access individual variable effect on CVR. Multivariable regression analysis was attempted with acute symptoms effects on CVR, which failed due to lack in collinearity between these variables between subjects.

1. Reliability and Reproducibility of the procedure: The test protocol involved seven stages as discussed earlier divided into three phases i.e., Baseline, Stimulus, and Hypocapnic. Data was recorded and collected at twenty-second intervals as recorded by TCD. Variations in a test protocol can occur in two planes i.e., between subjects changes (changes between different subjects for a test protocol against individual baseline) and within subject changes (changes within a subject for different phases through the protocol). The within cluster (Fixed Effects regression) is unnecessary, given the protocol required changes within different phases. To evaluate the reproducibility of this test, a series of analyses was done as follows to assess how similar the repetition of results of procedure are between subjects and within subjects during the different phases of the protocol. This was done in a 3-step sequential analysis.
    a. First, basic baseline means, standard deviation, between subject and within subject values were found for each indicator variable (Blood velocity, PI, RR, $ETCO_2$) for the three phases across its different stages for comparison (B1=B2=B3; S1=S2; H1=H2).
    b. Second, a longitudinal multilevel between subject variation statistical test was performed to access how similar these mean values are.
    c. Third, an interaction of these indicator variables with each other was analyzed to compare with previous literature i.e., interaction of blood velocity with PETCO2, PI and RR. This was done at group level, to exclude any influence of dependent variable changes on these values.
2. Data analysis included symptomatic versus asymptomatic group comparisons for CVR followed by addition of confounders. Subsequently, specific COVID factors and CVR changes was analyzed within symptomatic individuals (cases only).

Ethics and Funding

The current research was approved by The University of Texas Health Science Center at Tyler Institutional Review Board (IRB). This investigator-initiated study received a combined funding worth $7000 from Society of Vascular Ultrasound (SVU) and The UT Health Neurological Department. The SVU is a national level organization, adhered to promote research in areas requiring ultrasound. They issued an open grant $5000. The rest of the funding was supplied by the UT Health Neurological Department. We have no conflicts of interest to declare.

Chapter 4

RESULTS:

This study recruited 26 participants for testing CVR differences among symptomatic COVID for PCNS, against controls, using carbogen as stimuli and TCD. Three participants were excluded due to not meeting the inclusion criteria and two participants opted out after the discussion of the protocol. The 26 individual sample revealed 10 cases (38%) and 16 controls (62%). Twelve participants identified as male (46%), and 14 as female (54%). The ethnicity was predominantly White with 21 participants (81%) and rest of the sample included 2 participants of Hispanic origin (7.7%), a single Asian (3.9%) and 2 mixed-race individual (7.7%). There were 15 non-smokers (60%) and the rest had some form of smoking history (40%). Within the positive COVID cases, 6 individuals experienced COVID symptoms only once (60%), while 4 individuals had a symptomatic COVID positive test more than once (40%). The time when COVID symptoms were experienced was documented as during first quarter (January-April), second quarter (May-August), and third quarter (September-December) for the years 2020, 2021, and 2022. 2 individuals reported COVID symptoms 21 (See Table 1 and Table 2.).

Table 1: Demographics in Cases and Controls:

Demographics	Total sample	Cases	Controls
Sample	26	10 (38%)	16 (62%)
Age (years)			
Mean (SD)	23.73 (6.10)	24.19 (7.13)	23.4 (5.48)
Median (Min, Max)	21 (18,38)	21 (18,38)	21 (18,35)
Gender (n, %)			
Males	12 (46%)	4 (33.3%)	8 (66.6%)
Females	14 (54%)	7 (50%)	7 (50%)
BMI (n,%)			
Healthy Weight	13 (48.1%)	6 (46.2%)	7 (53.8%)
Overweight	8 (30.8%)	3 (37.5%)	5 (62.5%)

Obesity	5 (19.2%)	2 (40%)	3 (60%)
Race (n, %)			
White	21 (81%)	11 (52.3%)	10 (47.6%)
Non-White	5 (19%)	0	5 (100%)
Hispanic	2	0	2
Asian	1	0	1
Mixed race	2	0	2
Smoking History (n, %)			
Nonsmoker	15 (60%)	7 (46.7%)	8 (53.3%)
Smoker	11 (40%)	4 (36.4%)	7 (63.6%)
Cigarette Smoking		0	1
Vaping		3	4
Multiple*		1	1

(*) Multiple modes of smoking

Table 2: COVID cases only:

COVID HISTORY (CASES ONLY)	10	
COVID Once		6 (60%)
COVID more than once		4 (40%)
COVID exposure time/year (n, %)*		
(~Symptoms to test time in months)		
2020 1st Quarter (32 months)		2 (18.2%)
2020 2nd Quarter (28 months)		0
2020 3rd Quarter (24 month)		1 (9.1%)
2021 1st Quarter (20 months)		2 (27.3%)
2021 2nd Quarter (16 months)		3 (27.3%)
2021 3rd Quarter (12 months)		0
2022 1st Quarter (8 months)		1 (9.1%)
2022 2nd Quarter (4 months)		1 (9.1%)
2022 3rd Quarter		Testing
Time to test date		
Mean number of days (SD)		576.7 (261.8)
Median number of days (Min, Max)		610 (123-976)
Severity of symptoms (n, %)**		
Mild symptoms		5 (50%)
Moderate symptoms		4 (40%)
Severe symptoms		1 (10%)
Duration of symptoms (n, %)		
1-2 weeks		9 (90%)
3-4 weeks		1 (10%)

(*) 1st Quarter= January-April; 2nd Quarter= May-August; 3rd Quarter= September-December
(**) Mild: Initial symptoms of pneumonia/mild pneumonia, diarrhea, cough and fever, Moderate: Dyspnea, reduced oxygen saturation, Severe: Respiratory failure, sepsis, organ failure (hospitalized)

Reliability and reproducibility:

The test protocol involved 7 phases as discussed earlier. Data was recorded and collected at twenty-second intervals as recorded by TCD. To evaluate the reproducibility of the test, a series of analyses was done as follows.

Results reveal a consistency in the mean velocity differences between subjects across the baseline, stimulus, and hyperventilation stages with an approximate 13, 16, and 14 units of standard deviation respectively (Table 3). The table shows consistency for all individual parameters for each phase for each variable for mean, in-between subjects, and within-subject values. A statistical test was conducted to further assess the similarity between subjects for these values as a second step in analysis.

Table 3: **Comparison of Baseline phases, Stimuli phases, and Hypercapnic phases for Mean, Standard Deviation, Between subject variation and Within subject variation.**

Variable	**Phases**		
Baseline	**B1**	**B2**	**B3**
Blood Velocity			
Mean	59.84	54.70	53.81
Standard Dev	13.76 (34-109)	12.93 (32-76.5)	12.89(33.3-78.75)
Between	12.63 (39.15-87.25)	12.26 (32-76.5)	12.89 (33.3-78.75)
Within	5.53 (34.36-81.59)	5.63 (35.36-71.86)	4.71 (38.81-68.06)
Pulsatility Index (PI)			
Mean	0.99	0.94	1.11
Standard Dev	4.12 (0-10.1)	4.28 (0-6.9)	4.23 (0-6.6)
Between	0.98 (0-1.6)	0.27 (0-1.43)	1.3 (0-7.8)
Within			
Respiratory Rate (RR)			
Mean	14.38	13.80	13.77
Standard Dev	6.89 (0-30)	6.58 (0-36)	6.28 (0-27)
Between	4.85 (0-22)	4.76 (0-21)	5.09 (0-20)
Within	5.07 (-7.4-32)	4.84 (-1.2-37.79)	4.29 (-6.6-26.05)

Partial End-Tidal CO_2			
Mean	35.43	33.58	33.53
Standard Dev	10.61 (0-45.2)	8.40 (0-42.6)	9.29 (0-42.3)
Between	9.21 (0-42.6)	8.93 (0-40.9)	9.75 (0-40.73)
Within	6.08 (-3.42-61.88)	3.27 (14.69-55.25)	3.50 (2.33-39.93)

Stimulus (CO_2)	**S1**	**S2**
Blood Velocity		
Mean	70.33	69.52
Standard Dev	16.13 (30-118)	16.86 (35-116)
Between	15.17 (38.08-108)	15.82 (44.25-102.25)
Within	5.93 (34.91-84.34)	5.96 (40.43-85.44)
Pulsatility Index		
Mean	0.80	0.80
Standard Dev	0.19 (0-1.52)	0.18 (0-1.56)
Between	0.19 (0-0.97)	0.20 (0-1.02)
Within	0.13 (0.39-1.47)	0.12 (0.44-1.34)
Respiratory Rate		
Mean	11.29	12.12
Standard Dev	7.78 (0-68)	7.06 (0-45)
Between	3.97 (0-18.5)	5.71 (0-28)
Within	6.96 (-2.2-67.62)	5.69 (-2.2-43)
Partial End-Tidal CO_2		
Mean	37.59	37.50
Standard Dev	6.39 (0-44.9)	5.61 (0-47.5)
Between	7.91 (0-42.55)	7.96 (0-42.67)
Within	4.00 (14.85-57.15)	4.32 (5.00-55.81)

Hypocapnic	**H1**	**H2**
Blood Velocity		
Mean	47.93	46.29
Standard Dev	14.20 (24-100)	13.26 (23-93)
Between	13.71 (25-83)	12.94 (25.33-81.33)
Within	5.07 (34.93-64.93)	3.51 (33.96-57.96)
Pulsatility Index (PI)		
Mean	1.09	1.11
Standard Dev	0.35 (0-1.65)	0.38 (0-1.8)
Between	0.29 (0-1.49)	0.36 (0-1.53)
Within	0.19 (0.62-2.06)	0.14 (0.76-1.47)
Respiratory Rate (RR)		
Mean	42.22	36.83
Standard Dev	29.73 (0-117)	30.22 (0-113)
Between	25.28 (0-95)	24.22 (0-101)
Within	16.09 (0.22-84.22)	18.43 (-16.83-106.5)
Partial End-Tidal CO_2		

Mean	26.08	24.36
Standard Dev	9.05 (0-40.7)	9.60 (0-41.5)
Between	7.38 (0-37.67)	8.33 (0-36.7)
Within	5.36 (13.34-42.68)	4.93 (12.33-38.76)

The model showed no statistically significant interaction variation for variables between subjects adding to the homogeneity of the protocol across subjects. The goodness of fit for the model accounted for 93% of between subject variability with a probability of less than 0.01% (R^2=0.93, F=<0.01). The probability values for interaction of PI, RR and $PETCO_2$ are given in Table 4.

Table 4: Between subjects interaction variation (Statistical test for difference at phase level; Level-2).

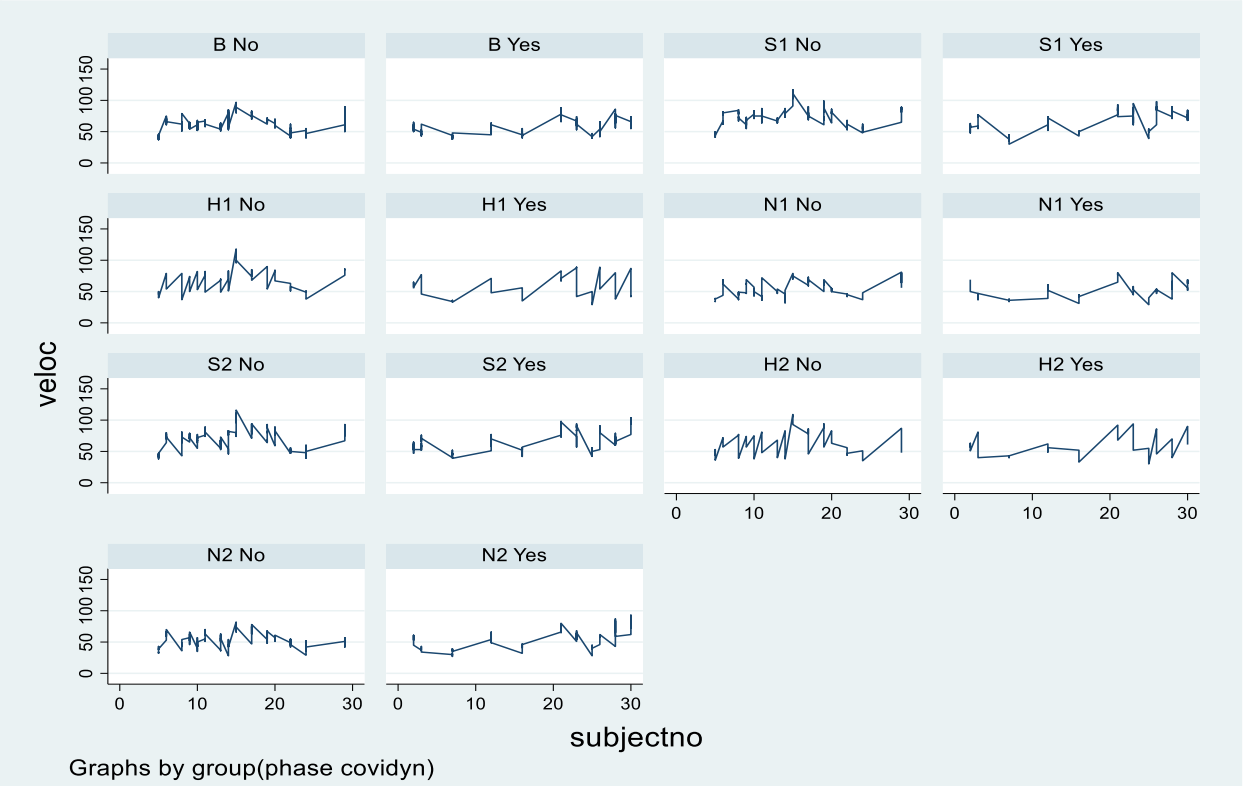
Variable	Slope (95% CI)	P-value
Pulsatility Index (PI)	-27.40 (-60.8-5.99)	0.08
Respiratory Rate	0.28 (-0.89-1.45)	0.51
Velocity	21.48 (-133.86-176.82)	0.69
End-Tidal CO_2	21.48 (-1.72-5.15)	0.21

The $PETCO_2$ had the maximum t-value (most interaction with velocity), against blood velocity with unit increase in velocity for every 0.62-unit change in $PETCO_2$. An inverse relation between velocity and PI is also observed as a decrease in PI value by 0.2 units for every 1 unit increase in velocity. The overall residual error for velocity is 13.63 (13.16-14.12) consistent with our baseline mean variation (Table 5)

Table 5: Mixed Multivariable Regression Analysis at Case/Control and Phase level (Case/Control; Level-3).

Variable	Slope (95% CI)	P-value
Velocity	41.07 (34.69-47.45)	0.00
Pulsatility Index (PI)	-0.23 (-0.46-0.01)	0.04
Respiratory Rate (RR)	-0.15 (-0.21-0.09)	0.00
End-Tidal CO_2 ($ETCO_2$)	0.62 (0.48-0.75)	0.00

A comparison graph of blood velocities for cases and controls for baseline, stimulus, and hypercapnic phases shows a similar line graph respectively (Graph 1). The visual graph shows a similar line graph between phases at case and control level for baseline phase (B No= N1 No= N2 No; B Yes= N1 Yes= N2 Yes). A similar line graph is observed for Stimulus and Hypercapnic phases as well (S1 No=S2 No; S1 Yes= S2 Yes) and (H1 No= H2 No; H1 Yes= H2 Yes). These consistencies in values indicate a relatively reliable and consistent procedure.



Graph 1. Mean velocity of cases versus controls within different phases of the protocol. (Graphical representation of velocities N1-N2, S1-S2, and H1-H2)
Y-axis: Subjects, X-Axis: Mean Blood Velocity.
Yes: COVID Positive (Cases); No: Controls
B/N: Baseline phase, S: Stimulus phases, H: Hypercapnic phase

Data Analysis:

CVR analysis for Symptomatic COVID positive versus controls:

A t-test analysis of equal variances for CVR between cases and controls showed no statistically significant differences among them (Table 6). A multiple regression analysis followed with potential confounders affecting CVR like age, weight, race, and smoking history. When controlled for confounders, the difference in CVR for cases versus control shown some differences. There was an increase in CVR by 3.8 units among cases as compared to controls (p=0.007, CI= 1.01-6.52). There was an increase in CVR by 6 units in overweight individuals as compared to normal Body Mass Index (BMI) (p<0.01, CI=3.80-9.10). Females had lower CVR values by 5 units compared to males (p<0.01, CI=8.3-2.94). Unit increase in age was associated with an increase in CVR by 0.31 units (p=0.003, CI= 0.10-0.52). Smoking status and race were not found statistically significant to CVR parameters (Table 7).

Table 6: T-test CVR and case control (Group Comparison)

Group	Mean CVR (SD)	95% CI	P-value
COVID Negative	58.90 (15.1)	50.88-66.93	0.65
COVID Positive	61.63 (14.4)	51.31-71.95	

Table 7: Regression analysis of CVR with confounders

Variable	Slope(95%CI)	P-Value	Referent
COVID Positive	3.77 (1.02-6.52)	0.007	
BMI			Underweight
Overweight	6.45 (3.8-9.1)	<0.01	
Obesity	-0.36 (-3.93-3.21)	0.844	
Gender	-5.61(-8.28-2.93)	<0.01	Males
Age	0.31 (0.11-0.52)	0.003	
Race	1.12 (-2.2-4.72)	0.495	White
Smoking History	1.79 (-0.76-4.34)	0.168	Non-Smoker

Covariate analysis of CVR among COVID positive individuals:

Other variables collected included Number of times experienced positive COVID symptoms, Severity of COVID symptoms, Duration of symptoms, Vaccination history and Time of year

experienced the symptoms. Duration of symptoms 1-2 weeks for 9 out of 10 cases within the sample, hence, was disregarded. Bivariate analysis for individual variables was performed (Table 8). The CVR was increased in individuals by 7.84 units with multiple symptomatic COVID positive experiences compared to a single exposure ($p<0.01$, CI=4.08-11.60). The CVR increased by 23.08 units for mild symptoms ($p<0.01$, CI=17.59-28.58) and 27.67 units for moderate symptoms ($p<0.01$, CI=22.17-33.16) compared to an asymptomatic positive COVID individual respectively. Vaccination history had no statistically significant effect on CVR ($p=0.17$). A list of variables for acute and chronic symptoms experienced during Covid exposure were collected but had minimal data. Dispersed data between subjects led to issues of collinearity, with scattered predictor variables not being able to independently predict the value of dependent variable. Bivariate analysis cannot be done due to multiple symptoms across individual subjects. Thus, a covariate analysis of acute symptoms to CVR had a statistically non-significant finding. (Table 9).

Table 8: Bivariate regression analysis of CVR among cases.

Variable	Slope(95%CI)	P-Value	Referent
COVID more than once	7.84 (4.08-11.60)	<0.01	COVID Once
Severity of Symptoms			Asymptomatic COVID
Mild	23.08 (17.59-28.58)	<0.01	
Moderate	27.67 (22.17-33.16)	<0.01	
Vaccination History	3.27 (-1.50-8.04)	0.178	COVID Vaccine Positive

Table 9: Covariate analysis for acute symptoms

Variable	Slope (95% CI)	SE	P-value
Fever or chills	-27.48 (-585.7-530.7)	43.9	0.64
Cough	-25.19 (-297.6-247.1)	21.4	0.44
Fatigue	63.6 (-438.6-565.8)	39.5	0.35
SOB*	29.7 (-330.6-390.0)	28.4	0.49
Muscle or Body aches	10.3 (-328.8-349.4)	26.7	0.77
Loss of smell	-1.2 (-273.5-271.2)	21.4	0.96
Congestion	-28.6 (-345.1-287.9)	24.9	0.45

Sore Throat	-0.8 (-299.2-297.5)	23.5	0.98

Chapter 5

Discussion

Bedside clinical monitoring for cerebral hemodynamic changes has been challenging for decades due to the intensive codependency of intrinsic factors such as cerebral flow, pressure, perfusion, and resistance. Isolating and improving any of these will improve the sensitivity of testing but in turn make the test complicated, heavily dependent on machinery, and expensive. The *in vivo* variation of the autoregulatory zone's slope/parameters, and dynamic characteristics within individual changes further complicates its application in forming a standard protocol. This has prevented a standard CVR test historically, with many researchers using a different model to reach reactivity (Aaslid, Lindegaard, Sorteberg, & Nornes, 1989; Fedriga & Czosnyka, 2021).

Although many researchers derive a CVR through various measures, an accurate read of the phenomenon remains challenging. The testing protocol in this study aims to initiate a simple bedside test for high-risk cerebrovascular LHC individuals and elicit a standard protocol along different phases of the protocol. The current protocol incorporates a dilatory (carbogen stimulus) as well as a hypercapnic (quick breathing) challenge to assess the overall change at the arteriole level. One of the objectives of the study was to assess the efficacy/reliability of TCD as a bedside tool and reproducibility of the current protocol. The single event longitudinal time data were time stamped and recorded for every 20 seconds. A comprehensive mean test to access an overview of the mean results for all indicator variables for different phases within the protocol was conducted first. This mean value was compared to its counterpart within the protocol. The values followed a set pattern with relatively similar values for each of baseline, stimulus and hypercapnic phases. On observation, the mean velocity for baseline phase was approximately 55 cm/sec, increasing to an approximate mean of 70 cm/sec in stimulus phase and dropping down to a relative 47 cm/sec in hypercapnic phase. This follows a set rhythm of a spike in blood flow

velocity on stimulus, followed by a drop in blood flow velocity on hypercapnia and return to baseline during the null phase. The standard deviation, between subject variation and within subject variation for these mean values for blood velocity had similar observational values on comparison for baseline, stimulus and hypercapnic phases. A similar finding was observed for all other indicator variables. The PI values remain around 1 for baseline phase, dropping to around 0.8 for stimulus phase and rising to 1.1 in hypercapnic phases. This pattern is consistent with the literature in its reciprocal effect to the blood flow velocity parameters and decreases by 0.23 units with an increase in velocity by 1 unit (Beishon, Haunton, Panerai, & Robinson, 2017). The $PETCO_2$ had the maximum t-value (most interaction with velocity), against blood velocity with unit increase in velocity for every 0.62-unit change in $PETCO_2$. The $PETCO_2$ showed a 5 mm/hg increase in stimuli compared to baseline and the hypercapnia on an average decreased by 10 mm/hg to baseline which is equivalent to a 20% drop of baseline Torr as suggested by the literature (1 Torricelli≈1 mm of Hg) (Rinsky, 2022). Most of these parameters had similar findings for mean, standard deviations, between subject and within subject variation parameters indicating a homogeneity of test results during phases for subjects. A fixed between effects model analysis with phase level panel data was performed to analyze further any statistical differences among these mean values for indicator variables. The model accounted for 93% of the variance for all values and found no difference statistically for the between subjects. The protocol showed consistencies in its results between different subjects indicating reproducibility. Therefore, reliability and efficacy of our procedure is good affirming our second objective.

The present study was performed to measure possible differences in CVR between asymptomatic vs symptomatic individuals with COVID history, our objective number one. There were no significant differences observed between these groups for CVR. However, when

regressed for CVR with known confounders, like gender, weight, age, race and smoking history, CVR was statistically significant between cases and controls. Symptomatic COVID positive individuals were found to have increased CVR values by 3.7 units compared to asymptomatic individuals. This means that there is an increased overall dilatory capacity of cerebral vessels among individuals with history of COVID symptoms compared to asymptomatic individuals. Though the study hypothesis of CVR difference between groups was shown, the results are contrary to my assumption of decreased reactivity among cases compared to controls. A possible explanation for this could be the sigmoidal nature of cerebral vessels in its relation to $PETCO_2$. According to the literature, the maximal dilation of vessels *in vivo* occurs within a range of 44-48 mm of Hg (Rinsky, 2022). In my effort to minimize machinery, the rebreathing technique of expired air from the attached bag fixed with a one-way valve was avoided and I was able to elicit a vasodilatory challenge at a mean peak of 5mm of Hg against the recommended 10 mm of Hg. The possible effect seen is initial dilation of vessels due to stimuli, and indicative of more readiness of this initial dilation among symptomatic COVID history individuals compared to asymptomatic individuals. This initial dilation along with total vasoconstriction in the hypercapnic phase elicits a total modulation reactivity capacity of cerebral vessels in both groups and differs significantly between each other (Azevedo & Castro, 2016; Castro & Azevedo, 2022; Christopher Kenneth Willie, 2014).

Gender, age, and BMI are known confounders for CVR as well as TCD measurements. My study included an age range of 18 to 40 years which is lower than most of the conventional CVR research within the literature. In my study, the difference between case and control groups in CVR is significant, though conflicting, with an increase in CVR in the cases rather than a

decrease. A similar finding of increased reactivity is observed with symptomatic COVID individuals by number of episodes and severity of symptoms.

Blood velocity has been known to decrease with age and females have been known to have physiologically higher blood velocity compared to males (Karnik et al., 1996; Kastrup, Thomas, Hartmann, & Schabet, 1997). CVR changes have been shown to decrease significantly in the later decades of life, starting mostly in the 4th decade of life. Factors such as fluctuations in estrogen and prostaglandins in females cause variations among blood velocity and CVR values (Karnik et al., 1996; Kastrup, Thomas, Hartmann, & Schabet, 1997). In our current study, when controlled for all other variables, CVR increased by 0.31 units for an increase in every year of life. Females had lower CVR values by 5 units compared to males contrary to usual findings of increased CVR values among females than men.

I also wished to investigate if there were any specific COVID associated neurological symptoms having any significant impact on CVR and if there is any association between symptoms to test date. The associated symptoms had very few observations and lacked any connection to CVR. A small sample size and dispersed observations within the sample led to the model not having enough observations to form collinearity between CVR and said observations.

Strengths and Limitations:

The study was successful in its effort to elicit CVR parameter differences between asymptomatic COVID versus symptomatic COVID individuals. One major limitation of the study was the sample size. A pre-test sample size calculation indicated a need of a minimum of 93 individuals to have a power of 0.8% for the current study. Another limitation of the study was its homogenous racial demographic. The study was predominantly of Caucasian background, one needs a much more heterogenous sample to be reflective of a community population. Due to the

infectious and widespread pandemic nature of COVID, it was difficult to isolate individuals uniquely as to having its effect due to infection. This is further exacerbated by vaccinated community, having positive titers, making it difficult to find true positive cases. The current study oversimplified differentiation of cases and controls based on symptomatic versus asymptomatic. In addition, individuals needed to report their symptoms in the questionnaire from memory, so the study is prone to recall bias.

One strength of the study was being able to elicit the reproducibility of the test in one of its simplest forms, paving the way for it to be a bedside non-invasive clinical test to help screen individuals at high risk. To avoid any complications for trial run of this assessment, individuals with prior history of any chronic medical condition or comorbidities were screened out before the test was conducted. Furthermore, the exogenous stimuli (carbogen) was reliable and reproducible. Having a relatively younger age group and excluding individuals with comorbidities helped me understand reactivity issues without the bias of these factors on reactivity. As a pilot study, this research was successful.

Conclusion:

The current study examined possible long-term complications of the cerebrovascular system by testing the vessels reactivity due to COVID, better understood today as Long Haul or Post COVID. CVR is a known parameter to screen individuals early for any possible cerebrovascular events in the future. The study showed a difference in CVR parameters between symptomatic COVID and asymptomatic/without COVID individuals. Though a small study, I was able to elicit a difference in reactivity among individuals with a symptomatic history with COVID. The use of Carbogen and minimal machinery helped in standardizing the protocol and its reproducibility as a clinical bedside tool. This difference shows that one can screen

individuals early to categorize them by PCNS status and help prevent a major cerebrovascular anomaly in the future. One objective of the study was to observe any change in CVR parameter following time. This would have helped one to understand any prospective changes in cerebral vasculature with COVID history. The low sample size limited my study in exploring this hypothesis.

Personal Statement:

This project was one of the biggest challenges of my life. One of the first challenges faced was to find appropriate funding for the project. This project helped me understand how to apply for grants, which is an essential skill for me to advance any of my future research projects. The second challenge was to get permission for the use of Carbogen. The Review board was apprehensive of the use of a medical gas on human subjects for an experiment. Getting an FDA exempt status helped me learn how to navigate through national control organization protocols and procedures. Finding companies who produced the required medical gas and placing a formal order for a medical gas and its wait time was the next hurdle. Being able to explain potential subjects and recruiting them for the test was no easy task either. Formulating a schedule to fit in all the necessary individuals required a different skill set. Lastly, learning a new statistical software (STATA) to run the required statistical tests is a new skill developed. I am most grateful for learning it because I will potentially be using it for most of my research in the future.

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ABBREVIATIONS

Angiotensin-converting enzyme 2 (ACE2)
Carbon dioxide (CO_2)
Carotid Revascularization and Medical Management for Asymptomatic Carotid Stenosis Trial (CREST-II)
Carotid Revascularization and Medical Management for Asymptomatic Carotid Stenosis–Hemodynamics trial (CREST-H)
Case fatality rate (CFR)
Central Nervous System (CNS)
Cerebral Autoregulation (CA)
Cerebral Blood Flow (CBF)
Cerebral Perfusion Pressure (CPP)
Cerebral Vasomotor Reactivity (CVR)
Cerebrovascular anomaly (CVA)
Damage-associated molecular patterns (DAMPs)
Disseminated Intravascular Coagulopathy (DIC)
End-Tidal CO_2 ($ETCO_2$)
Food and Drug Administration (FDA)
Guillain-Barré syndrome (GBS)
Institutional review board (IRB)
Interleukin (IL)
International Committee on Taxonomy of Viruses (ICTV)
Left Middle Cerebral Artery (LMCA)
Long-Haul COVID (LHC)
Magnetic Resonance Imaging (MRI)
Mean flow velocities (MFV)
Middle Cerebral Artery (MCA)
National Institute for Health and Care Excellence (NICE)
National Institutes of Health Stroke Scale (NIHSS)
Neuropilin (NRP)
Partial End-Tidal CO_2 (PETCO2)
Peripheral Nervous System (PNS)
Polymerase Chain Reaction (PCR)
Post-Acute Sequelae of COVID-19 (PASC)
Post-COVID Neurological Syndrome (PCNS)
Posterior Reversible Encephalopathy Syndrome (PRES)
Pulsatility Index (PI)
Respiratory Rate (RR)
Royal College of General Practitioners (RCGP)
Scottish Intercollegiate Guidelines Network (SIGN)
Severe acute respiratory syndrome-related coronavirus (SARS)
Southwestern Cerebral Circulatory Dynamics (SCCD)
Texas Department of State Health Services (DSHS)
The Asymptomatic Carotid Surgery Trial-2 (ACST-2)
The Center for Disease and Control (CDC)

The National Institute of Health (NIH)
Transcranial Doppler (TCD)
Transmembrane protease serine 2 (TMPRSS2)
World Health Organization (WHO)

APPENDIX-1

Inclusion/ Exclusion criteria:

1. **Presence of COVID-19 signs and symptoms on the day and three months prior to testing date (check below)**
COVID -19 symptoms:

i. Fever or chills
ii. Cough
iii. Shortness of breath or difficulty breathing
iv. Fatigue
v. Muscle or body aches
vi. Headache
vii. New loss of taste or smell
viii. Sore throat
ix. Congestion or runny nose
x. Nausea or vomiting
xi. Diarrhea
xii. Trouble breathing
xiii. Persistent pain or pressure in the chest
xiv. New confusion
xv. Inability to wake or stay awake
xvi. Pale, gray, or blue-colored skin, lips, or nail beds, depending on skin tone

2. **COVID Testing history:**

i. Have you ever been **lab tested positive** for COVID-19: Yes___ No___
ii. **Non-tested but had to quarantine due to close contact with COVID-19 positive individual**:

a. Please mention if you have never officially lab tested for COVID-19, yet **had positive signs and symptoms"** (above mentioned symptoms) due to close contact with lab positive COVID-19 individual and had to undergo subsequent quarantine:

b. **Did not had positive signs and symptoms**, had contact with positively tested individual and underwent quarantine:
iii. **Never had any signs nor symptoms, nor tested positive**: Yes___ No___

2. **Mention if you have history of any of the following:**

i. Diabetes (Type 1): Yes___ No___
ii. Diabetes (Type 2): Yes___ No___
iii. Treated hypertension (diagnosis/at least one current prescription drug use): Yes___ No___
iv. Asthma: Yes___ No___
v. Bronchiectasis: Yes___ No___
vi. Bronchitis/Chronic Cough: Yes___ No___
vii. Chronic Obstructive Pulmonary Disease (COPD): Yes___ No___

viii. Asbestosis/ Silicosis/ Sarcoidosis: Yes___ No___
ix. Cystic Fibrosis: Yes___ No___
x. Pleurisy: Yes___ No___
xi. Pneumonia (requiring extended hospitalization 5 days and more): Yes___ No___
xii. Pulmonary Embolism/Deep vein thrombosis: Yes___ No___
xiii. Pulmonary Hypertension: Yes___ No___
xiv. Sleep Apnea: Yes___ No___
xv. Tuberculosis: Yes___ No___
xvi. Arthritis (Rheumatoid/ Psoriatic/Osteoarthritis): Yes___ No___
xvii. Gout: Yes___ No___
xviii. Ankylosing spondylitis: Yes___ No___
xix. Arrhythmias/Abnormal heart rhythms: Yes___ No___
xx. Marfan syndrome/ Aorta disease: Yes___ No___
xxi. Congenital heart disease: Yes___ No___
xxii. Coronary artery disease (narrowing of the arteries): Yes___ No___
xxiii. Heart attack: Yes___ No___
xxiv. Heart failure: Yes___ No___
xxv. Heart muscle disease (cardiomyopathy): Yes___ No___
xxvi. Heart valve disease: Yes___ No___
xxvii. Pericardial disease: Yes___ No___
xxviii. Peripheral vascular disease: Yes___ No___
xxix. Rheumatic heart disease: Yes___ No___
xxx. Stroke: Yes___ No___
xxxi. Vascular disease (blood vessel disease): Yes___ No___
xxxii. Bleeding in brain (ICH): Yes___ No___
xxxiii. Alzheimer's disease/Dementia/Mild Cognitive Inhibition: Yes___ No___
xxxiv. Guillain-Barré Syndrome: Yes___ No___
xxxv. Parkinson's disease: Yes___ No___
xxxvi. Dystonia (involuntary muscle contractions): Yes___ No___
xxxvii. ALS (Amyotrophic Lateral Sclerosis or Lou Gehrig's disease): Yes___ No___
xxxviii. Huntington's disease: Yes___ No___
xxxix. Neuromuscular disease: Yes___ No___
xl. Multiple sclerosis: Yes___ No___
xli. Epilepsy/Seizures: Yes___ No___
xlii. Chronic Kidney Disorder: Yes___ No___
xliii. Cancer (benign/malignant): Yes___ No___

3. **<u>Drugs history: Please indicate if you are taking any of the following medications:</u>**

Beta-blockers: Yes___ No___
anti-arrhythmic (e.g., Norpace): Yes___ No___
Anti-depressants: Yes___ No___
Digitalis/digoxin: Yes___ No___
Blood thinners (e.g., Warfarin, Heparin): Yes___ No___
Tamoxifen: Yes___ No___
Evista (raloxifene): Yes___ No___

Corticosteroids (e.g., prednisone): Yes___ No___
Adrenaline/epinephrine: Yes___ No___
Mention if any other medication used on a regular basis:

4. **Please specify if any of the following applies to you:**

i. You are pregnant now: confirmed by a doctor or taken a self-pregnancy test and tested positive : Yes___ No___
ii. You are uncertain you might be pregnant and is planning to take a test soon: Yes___ No___
iii. Does not apply to you: _____

5. **FOR LAB PURPOSE ONLY, DO NOT ANSWER:**

i. Finding of abnormal blood pressure reading on testing date (>90-130< mm Hg).
ii. Uncontrolled Blood pressure fluctuation while testing
iii. Failure to adhere/comply with mask or gas during the procedure.
iv. Age above and below inclusion criteria
v. Non-English speaking

APPENDIX-2
Questionnaire

Demographics:

1. **Subject Id number: _____**

2. **Age: ____**

3. **Sex:**

Male: ____
Female: ____
Other: _____

4. **Race:**

Caucasian: ____
African American: ____
Hispanic: ____
Native American: ____
Asian: _____
Other: _____

5. **Blood Pressure/Pulse: _____**

6. **BMI: _____**

7. **Vaccination history:**

Vaccinated: Yes___ No___
Non-Vaccinated: Yes___ No___

8. **Number of times tested as infected (positive lab test):**

Once: Yes___ No___
More than once: Yes___ No___

*****Answer question 9-13 in accordance with your "FIRST EXPOSURE, FIRST TIME TESTED POSITIVE":**

9. **COVID-19 history:**

Choose roughly the duration when you had COVID positive history (if lab tested positive) (Approximate start date):

January-April (2019): Yes___ No___
May- August (2019): Yes___ No___
September-December (2019): Yes___ No___
January-April (2020): Yes___ No___
May- August (2020): Yes___ No___
September-December (2020): Yes___ No___
January-April (2021): Yes___ No___
May- August (2021): Yes___ No___
September-December (2021): Yes___ No ___
January-April (2022): Yes__ No ___

10. **What were your predominant COVID-19 symptoms (Acute phase)?**

Fever or chills: ___
Cough ___
Fatigue ___
Shortness of breath or difficulty breathing ___
Muscle or body aches ___
Headache ___
New loss of taste or smell ___
Sore throat ___
Congestion or runny nose ___
Nausea or vomiting ___
Diarrhea ___
Trouble breathing ___
Persistent pain or pressure in the chest ___
New confusion ___
Inability to wake or stay awake ___
Pale, gray, or blue-colored skin, lips, or nail beds, depending on skin tone ___
Others (if any) _____

11. **How long did the signs and symptoms of acute phase last:**

1-2 weeks: Yes___ No___
3-4 weeks: Yes___ No___
5-6 weeks: Yes___ No___
7-8 weeks: Yes___ No___
9-10 weeks: Yes___ No___
11-12 weeks: Yes___ No___
12 weeks and more: Yes___ No___

12. **Severity of the acute phase:**

Testing positive but No symptoms (Asymptomatic): Yes___ No___
Mild symptoms but no treatment required (functional for daily life): Yes___ No___
Moderate symptoms (bedridden): Yes___ No___

Severe symptoms (pain and not functional): Yes___ No___
Hospitalized: Yes___ No___

13. ***Long COVID or PCNS history (post-acute phase):***

Please specify if you felt any of these three months after the acute phase infection:
(*** Please remember, the following need be answered yes, only if it is of new onset post COVID infection and without a prior chronic history of it).

Fatigue: Yes___ No___
Muscle weakness/joint pains: Yes___ No___
Sleep difficulties/insomnia/sleep disorders: Yes___ No___
Impaired concentration (cannot concentrate at times): Yes___ No___
Cognitive impairment (change in remembering things post infection): Yes___ No___
Polyneuropathy (tingling/numbness/difficulty using arms or legs): Yes___ No___
Burning feet pain: Yes___ No___
Inability to feel pain: Yes___ No___
Extreme sensitivity to touch: Yes___ No___
Heat intolerance/flushing: Yes___ No___
Dysautonomia (lack of control in movements): Yes___ No___
Difficulty walking: Yes___ No___
Loss of smell (anosmia): Yes___ No___
Ageusia (loss of taste): Yes___ No___
Alopecia (hair loss): Yes___ No___
Tinnitus (ringing in the ear): Yes___ No___
Erectile dysfunction: Yes___ No___
Seizures: Yes___ No___
Chronic Headache (without prior history, of minimum 3 day or more): Yes___ No___
Dizziness: Yes___ No___
Asthenia (lack of energy/not able to do things you did normally before infection): Yes___ No___
Depression/suicidal thoughts: Yes___ No___
Blurry vision: Yes___ No___
Impaired visual acuity (reduced/loss of vision): Yes___ No___
Eye pain: Yes___ No___
Xeropthalmia/sicca symptoms (dry eye/scratchy eyes): Yes___ No___
Exanthema (skin rash): Yes___ No___
Blisters: Yes___ No___
Chest pain: Yes___ No___
Difficulty breathing: Yes___ No___
Nasal congestion: Yes___ No___
Sneezing/coryza: Yes___ No___
Palpitations: Yes___ No___
Diarrhea/vomiting: Yes___ No___
Dyspepsia: Yes___ No___
Gastrointestinal symptoms: Yes___ No___

14. ***Social:***

Smoking status: Please check the following that apply
Non-smoker:
Former smoker:
Light smoker (<10 cigarettes/day):
Moderate smoker (10-19 cigarettes/day):
Heavy smoker (≥20 cigarettes/day):
Chewing Tobacco: Yes___ No ___
Vaping: Yes___ No___

For the following answer according to your memory of last 7 days for approximately 20 minutes or more (rough analogous IPAQ-S):
a. How many days did you do vigorous physical activities (like heavy lifting, digging, aerobics, or fast bicycling)?
1-2 days/week:
3-4 days/week:
5-7 days/week:
No vigorous physical activities:
b. moderate physical activities (like carrying light loads, bicycling at a regular pace, or doubles tennis)
1-2 days/week:
3-4 days/week:
5-7 days/week:
No moderate physical activities:
c. how many days did you walk for at least 10 minutes at a time:
1-2 days/week:
3-4 days/week:
5-7 days/week:
No minimal physical activity:

15. ***Family History: Please specify if any of your family members have a known history of:***

Stroke: Yes___ No___
Bleeding in brain (ICH): Yes___ No___
Brain tumor: Yes___ No___
Alzheimer's disease/Dementia/Mild Cognitive Inhibition: Yes___ No___
Guillain-Barré Syndrome: Yes___ No___
Parkinson's disease: Yes___ No___
Dystonia (involuntary muscle contractions): Yes___ No___
ALS (Amyotrophic Lateral Sclerosis or Lou Gehrig's disease): Yes___ No___
Huntington's disease: Yes___ No___
Neuromuscular disease: Yes___ No___

Multiple sclerosis: Yes___ No___
Epilepsy/Seizures: Yes___ No___
Mention if any neurological issues not listed above: Yes___ No___
Mention if any other general chronic conditions:

APPENDIX-3

The group has published 8 articles in the Journal of Neuroimaging and 6 articles in the Journal of Vascular Ultrasound. 10 papers were orally presented at the national meetings of these organizations. The most recent clinical research involves using transcranial Doppler to detect foramen ovale patent easily and reproducibly in cryptogenic stroke and TIA patients and CD in Code Stroke and post-mechanical embolectomy patients. In 2007, TCD was added to the CODE STROKE Protocol at ETMC-Tyler. From 1989 until the present, he has been involved in teaching cerebral hemodynamics, consulting with numerous manufacturers, and performing the technical component of 15,000 neurovascular examinations.

Current interests and ongoing research:

The failure of conscious sedation TEE to detect all patent foramen ovales.

Ultrasound-aided thrombolysis in acute MCA stenosis.

The patent foramen ovale as a cause of cryptogenic CVA in trauma and SAH patients.

Vasomotor reactivity measurements to detect SAH patients at risk for vasospasm.

Vasomotor reactivity measurements in carotid artery disease with concurrent basilar artery stenosis or occlusion.

Emboli detection in the asymptomatic carotid stenosis patient.

Measuring the size of a PFO to augment selection of patching candidates

SCCD has participated by submitting patients to the WASID trial, the CLOTBUST 1 trial and the Swiss PFO.

APPENDIX-4

THE UNIVERSITY OF TEXAS
Health Science Center at Tyler

The Center for Clinical Research
11937 US HWY 271
Tyler, TX 75708

INFORMED CONSENT FORM
AND
INFORMATION ABOUT

Efficacy of Transcranial Doppler (TCD) assessment of Cerebral Vasomotor Reactivity (CMVR) and Pulsatility Index (PI) using CO2 stimulus as an initial measure of Neurological Impact in Long COVID-19 or post-COVID-19 Neurological Syndrome (PCNS)

Principal Investigator: Musharaf Mohiuddin, MBBS, MPH, MHS **After hour's pager:**

_____ _____
Subject ID MRN

You are being offered an opportunity to participate in a research study that is supported by the UT Health Neurology department (sponsor) who is funding this study. The study Sponsor provides funding to cover some or all of the costs of conducting this research.

Before you agree to volunteer to take part in this research study, it is very important that you understand the purpose of the study and the nature of the tests and procedures you will be asked to undergo. Please read this Informed Consent form carefully and take your time making a decision about whether to participate. As the researchers discuss this Informed Consent form with you, please ask him/her to explain any words or information that you do not clearly understand. The purpose of the study, risks, inconveniences, discomforts, and other important information about the study are listed below. If you decide to participate, you will be given a signed copy of this form to keep.

You are being asked to take part in this study because you belong to the age group being investigated, with/without prior history of COVID infection.

Purpose and Background
The purpose of this research study is to investigate if the Transcranial Doppler Cerebral Vasomotor Reactivity assessment procedure, a non-invasive (not requiring the introduction of instruments into the body) brain imaging technique, can help identify if there is any long-term effects from the COVID virus on the brain and its blood supply. Cerebral vasomotor reactivity (CVMR) is a mathematical calculation done based on readings of an individual's brain blood flow

velocity/speed numbers. This brain blood velocity is captured by the Transcranial Doppler (TCD) scan machine. If CVMR calculation is reduced, it is a marker or indication of poor brain blood vessel health and has been associated in the long run for brain and nerve issues. This study procedure will compare the blood supply/CVMR of individuals who had COVID infection against individuals who did not have the infection. This will help understand if there are any long-term, unseen, and unnoticed damage to the brain and its blood supply due to prior infection with the coronavirus. This study does not reveal any immediate/at present brain abnormalities or current issues you can possibly have. It helps to understand and be better prepared for any complications that a person potentially may or may not face in the future in terms of any neurological problems through a cost effective and painless procedure. Even if the findings are positive, it is still not certain he or she will have issues associated with poor brain blood vessel health..

You will be one of approximately 40-80 subjects in this research study.

Procedures

If you volunteer to take part in this research study, and the study doctor has determined that you may be eligible to participate, you will be asked to sign this Informed Consent form and will undergo the tests and procedures outlined below. The tests and procedures outlined below will be performed at a single clinic visit. This procedure is being done solely for the purpose of this study.

You will be given a questionnaire to complete. You will be informed on the details of the procedure below.

The procedure: A basic neurological assessment (cognitive testing, gait, balance and eye movements) and blood pressure are recorded. A gel will be applied to the side of your temple and a probe is placed on it. You will be asked to breathe normally (room air with a mask on to get used to it) for a period of 10 minutes. Once you reach a relatively constant partial pressure of end-tidal carbon dioxide (PETCO2) measure on the monitor (i.e., the carbon dioxide content in your exhaled breath), an automatic calculated mean baseline blood flow velocity (MBFV - i.e., speed at which your blood is moving through your brain artery), Respiratory rate (i.e., number of breaths you are taking per minute) and Pulsatility Index (PI) (i.e., difference in your peak and lowest blood pressure) are recorded. You will be asked to breathe a gas called Carbogen (95% carbon dioxide and 5% oxygen) and a target of +10mm Hg from baseline is targeted (2-3 minutes). Your normal $PETCO_2$ is between 34-44 mm Hg and with gas stimulus we increase it from your normal to +10mm Hg. Sensations of air hunger (feeling out of breath like after intense exercise) and hot flashes (a generalized feeling of warmth) is expected during gas stimulation. This is followed by No gas (stimulus) period where you will be asked to rebreathe the expired air through the mask for 2-5 minutes and wait for $PETCO_2$ (As explained above) to restabilize.

Once your $PETCO_2$ stabilizes on the monitor during the no gas period, the gas stimulus is re-started (you will breathe in Carbogen gas again) to target $PETCO_2$ level of +10mm Hg to exhaust the vasodilation capacity/reserve. Your blood pressure will be recorded at the end of this procedure and the mask is removed. You will be monitored additionally for 5-10 minutes while you breathe normal air in the waiting area. -

Length of Study and Number of Visits

A single visit of approximately 45-60 minutes.

Discomfort and Risks
The procedure can cause:
a. Irritation when wearing the mask or discomfort on inhaling gas, temporary discomfort due to high-pressure oxygen and carbon dioxide administration.
b. Sensations of air hunger (feeling of out of breath like after intense exercise) and hot flashes (a generalized feeling of warmth) is expected during gas stimulation.
c. Acute stimulation with gas (carbogen) above 50-60 mm hg of gas is found to be uncomfortable.
d. Shortness of breath, headache, and dizziness were found in 1 out of 10 individuals only during the stimulus administration and ceased after end of stimulus within a couple of minutes.
e. Temporary increase in blood pressure (will be monitored).
f. The procedure may induce anxiety, restlessness, and breathlessness. Rare occasions have been reported of syncope (fainting), hallucinations, and delirium (confusion) for a short time after the procedure.

Benefits
The above procedure may help identify people in the population who are at high risk from prior COVID infection. It will help to be better prepared for precautionary measures and may potentially help prevent brain blood issues (stroke, bleeding, neurodegeneration) earlier in the event of abnormal findings. The community might benefit from inexpensive early screening, bedside diagnosis, and prevention of brain issues.

Alternative Therapies
You have the alternative of not participating in this study. The study is investigative in nature of assessing use of doppler imaging in COVID survivors. There is no alternative therapy for this study. This is not a treatment study.

Cost and Compensation
A compensation of $50.00 will be provided for participation in the study. Compensation will only be provided if you complete your participation in the study. You will not be charged for any services or procedures rendered.

<u>Important Payment and Tax Information</u>
The U.S. Internal Revenue Service (IRS) considers payments received for participation in research as income. UTHSCT is required to report payments of $600 or more in a calendar year to the IRS. However, it is your responsibility to report all income, regardless of the amount, to the IRS on your annual federal tax return.

Disclosure of your Social Security Number (SSN) is required in order for UTHSCT to report miscellaneous income, as mandated by Federal law. Further disclosure of your SSN is governed by the Public Information Act (Chapter 552 of the Texas Government Code) and other applicable laws.

UTHSCT, as a State Agency, is not allowed to make any payments to you for your participation in this research if the Texas State Comptroller has issued a "hold" on all State payments to you. Such a "hold" could result from your failure to make child support payments or pay student loans, etc. If this occurs, UTHSCT will be allowed to pay you for your taking part in this research after

you have made the outstanding payments and the State Comptroller has issued a release of the "hold".

Compensation for Injury or Illness Related to Study Participation
In the event of an injury or illness as a direct result of participation in this research study, your study doctor will assist you in receiving appropriate health care, including first aid, emergency treatment and follow-up care either at The University of Texas Health Science Center at Tyler or another appropriate health care facility. If medical costs are incurred, your insurance provider may be billed. UTHSCT will not provide further compensation beyond that which is listed in this informed consent form.

By signing this form, you will not lose any of your legal rights or release the Sponsor, the study doctor, the study staff, or the study site from liability for negligence or intentional misconduct. If you believe you have experienced any study related illness, adverse event, or injury, you must notify the study doctor as soon as possible. Your study doctor will discuss with you the available medical treatment options. Your social security number (SSN) may be requested if you are injured and you are a Medicare beneficiary.

Confidentiality
Your participation in this research study will be kept confidential in accordance with applicable law; however, your records related to this study may be disclosed as required by law for purposes explained in this Informed Consent (under "Confidentiality" and "Authorization to Use and Disclose Protected Health Information"). This access may involve inspection and copying of confidential study related records which identify you by name. Therefore, absolute confidentiality cannot be guaranteed. If the results of this study are published or presented at meetings, you will not be identified.

<u>Authorization to Use and Disclose Protected Health Information</u>
During your participation in this research study, the study doctor and study staff will collect or create personal health information about you (for example, medical histories and results of any tests, examinations or procedures you undergo while in the study) and record it on study documents. The study doctor will keep this personal health information in your study-related records (that we will refer to as "your study records"). In addition, the study doctor may obtain, and include in your records, information regarding your past, present and/or future physical or mental health and/or condition. The study doctor may ask you to sign a separate authorization to obtain some or all of your medical records from your doctor. Your study records may include other personal information (such as social security number, medical record numbers, date of birth, etc.), which could be used to identify you. Health information that could identify you is called "Protected Health Information" (or "PHI").

Under federal law (the "Privacy Rule"), your PHI that is created or obtained during this research study cannot be "used" to conduct the research or "disclosed" (given to anyone) for research purposes without your permission. This permission is called an "Authorization". Therefore, you may not participate in this study unless you give your permission to use and disclose your PHI by signing this Authorization.

By signing this Authorization, you also are agreeing to allow the study doctor to disclose PHI to the following described below:

- The University of Texas Health Science Center at Tyler employees involved in this study
- The University of Texas Health Science Center at Tyler Institutional Review Board (IRB – a group of people who strive to protect the rights of subjects)
- Local, state and federal agencies (such as the Office for Human Research Protections and the U.S. Food and Drug Administration) when required by law.

Except for the disclosures described above, your PHI will not be shared with others unless required by law. If your PHI is given to the parties listed above and/or to others who are not required to comply with the federal law, your PHI will no longer be protected by this law and could possibly be used or disclosed in ways other than those listed here.

You have a right to see and make copies of your PHI. You are agreeing, however, by signing this Informed Consent form, not to see or copy some or all of your PHI until the sponsor has completed all work related to this study. At that time, you may ask to see your records.

You have a right to revoke your Authorization at any time. If you revoke it, your PHI will no longer be used for this study, except to the extent the parties to the research have already taken action based upon your Authorization or need the information to complete analysis and reports for this research. To revoke your Authorization, you must write to the study doctor, stating that you are revoking your Authorization to Use and Disclose Protected Health Information. If you revoke this Authorization, you will not be allowed to continue to be in this study.

Whom to Contact for Questions
If you have any questions about the research, or in the case of injury or illness resulting from the research, please contact Musharaf Mohiuddin at 903-944-4338.

If you have additional questions during the course of this study about your rights as a research subject, you may address them to The University of Texas Health Science Center at Tyler Institutional Review Board (IRB) Office at (903) 877-7632.

Participation
Your participation in this study is voluntary. You can discontinue your participation at any time before or during the procedure.

Right to Withdraw
You may withdraw from participation in the study at any time without penalty or loss of benefits. However, if you decide to stop participating in the study, we encourage you to tell the researchers.

Your participation may be terminated at any time by the PI, doctor or hospital authorities if you are found to be not eligible based on inclusion/exclusion criteria, failure to comply by procedural protocols, or any event wherein your participation goes against the interest of research or might be suggestive of any possible adverse effect or finding that could harm you.

New Findings
Any new findings developed during the course of your participation in the study, which may be related to your willingness to participate, will be provided to you. If you choose to continue participation, you may have to sign a new Informed Consent to continue.

Statement of Consent

This research study has been explained to me and I have had an opportunity to read this consent form and have all of my questions answered. I have been informed that I may leave the study at any time without affecting my medical care and the Sponsor or my doctor may withdraw me from the study without my consent. I understand that becoming a research volunteer does not automatically make me a patient at The University of Texas Health Science Center at Tyler. My participation is limited to the research study only. I also understand that the study does not cover expenses related to medical care that are not related to study specific procedures. I freely agree to take part in this research. A signed copy of this consent form will be given to me.

_____	_____	_____
Printed Name of Subject	Signature	Date

Statement of Person Obtaining Consent

I have carefully explained to the subject the nature of the study. I hereby certify that to the best of my knowledge the subject signing this consent form understands clearly the nature, demands, risks and benefits involved in participating in this study. A medical problem or language or educational barrier has not prevented a clear understanding of the subject's involvement in this study.

_____	_____	_____
Printed Name of Person Obtaining Consent	Signature	Date

Appendix -5
Recruitment Flyer

UTTyler
THE UNIVERSITY OF TEXAS AT TYLER

APPROVED
04/27/2022
IRB# 2022-008
BY THE UTHET/UTHSCT/UT TYLER INSTITUTIONAL REVIEW BOARD

Post-COVID-19, Is your brain healthy? Is your brain receiving adequate blood supply?

Researchers at the UT Tyler and UT Health hospital are investigating a novel method to determine your brain health post-COVID-19. The test requires breathing modified air mixture and non-invasive image testing.

If you are between the **age of 18 to 40 years** old and have **prior exposure of COVID-19 three months prior to imaging date**, you may be eligible for this research study. If you **never had COVID-19** and is between the ages of 18 to 40 years old, you may still be eligible to participate.

Participant Reimbursement : $50!!!

To determine if you qualify and further questions, please contact Dr. Musharaf Mohiuddin at 903-944-4388 or email: mmohiuddin@ uttyler.edu

This project is supported with a gift from the UT Health *Neuro-department and Society for Vascular Ultrasound (SVU)*

UTHealth Tyler
SVU
SOCIETY FOR VASCULAR ULTRASOUND
Research - Education - Advocacy

Appendix -6
IRB Approval

UTHealth East Texas

UTHealth
The University of Texas Health Science Center at Tyler

DATE: 04/27/2022

TO: Musharaf Mohiuddin, MBBS, MPH
700 Olympic Plaza
Suite 906
Tyler, TX 75701

SUBMISSION TYPE:	Initial Review
PROTOCOL NUMBER:	2022-008
PROTOCOL TITLE:	Efficacy of Transcranial Doppler (TCD) assessment of Cerebral Vasomotor Reactivity (CMVR) and Pulsatility Index (PI) using CO2 stimulus as an initial measure of Neurological impact among young in Long COVID-19 or post-COVID-19 Neurological Syndrome (PCNS)
IRB ACTION:	**APPROVED**
APPROVAL DATE:	04/27/2022
EXPIRATION DATE:	04/26/2023
REVIEW TYPE:	Full Board Review
IRB MEETING DATE:	04/27/2022

CONTINUING REVIEW INTERVAL: **12**

Thank you for your Initial Review Submission for the above-referenced study. The UT Health East Texas Institutional Review Board has APPROVED your submission by ***Full Board Review.***

Items Submitted for Review:

- ***IRB Initial Review Submission Form***
  - ***Alexander CITI HSR certificate exp. 2022.05.05.pdf (Other)***
  - ***Alexander CV 2022.pdf (Investigator/Research Team CV or Resume)***
  - ***Data sheet-TCD 2022.04.12.xlsx (Data Collection Tools)***
  - ***Informed Consent-TCD Carbogen final.doc (Consent Form)***
  - ***Informed Consent-TCD Carbogen 2022.04.11 v2 draft clean final.doc (Consent Form)***
  - ***IRB 2022-008 Initial Review Determination--Table 02 23 2022 mtg.docx (Determination Letter)***
  - ***IRB 2022-008 Initial Review Determination--Table 02 23 2022 mtg.pdf (Determination Letter)***
  - ***Mohiuddin CITI HSR certificate exp. 2024.06.16.pdf (Other)***
  - ***Mohiuddin CV 2022.docx (Investigator/Research Team CV or Resume)***
  - ***Plotkin CV 2022.docx (Investigator/Research Team CV or Resume)***
  - ***Plotkin license exp. 2023.08.31.pdf (Other)***
  - ***Project Summary Form 2022.04.11 IRB.doc (Protocol)***
  - ***Questionnaire- TCD Carbogen 2022.01.27.docx (Data Collection Tools)***
  - ***Recruitment Flyers_TCD.docx (Patient Recruitment Materials)***

Institutional Review Board Office
1100 East Lake Street, Suite 330, Box-14
Phone: 903-877-7632
Email: irb@uthct.edu

- ***Research Proposal TCD CVMR.docx (Protocol)***
- ***Research Proposal TCD CVMR.docx (Other)***
- ***Study Protocol 2022.04.12 final version.docx (Other)***
- ***tcd data sheet.xlsx (Data Collection Tools)***

Research Team:
Musharaf Mohiuddin, MBBS, MPH - Investigator
Christopher Herrick, BS, CCRC - Coordinator
Debbie Fielder - Clinical Research Director
George Plotkin, MD - Co-Investigator
Thomas (Howie) Alexander, BS - Co-Investigator

All research must be conducted in accordance with this approved submission. Any changes to the research must be reviewed and approved by the UT Health Science Center/UT Health East Texas Institutional Review Board prior to implementation, except when necessary to eliminate an apparent immediate hazard to the subject.

You are reminded that you must apply for, and undergo review, and be granted continued IRB approval for this study before the study expiration date in order to be able to conduct your study in an uninterrupted manner. If you do not receive approval before this date, you must cease and desist all research involving human subjects, their tissue, and their data until approval is granted. However, changes can be implemented if they are in the best interest of the subject due to safety evaluations or eliminating/reducing risks to them. The determination of "best interest of the subject" must be made by the IRB. Alternatively, if your study has concluded please complete the "Study Closure Form" and forward to the UT Health Science Center at Tyler/UT Health East Texas IRB Office.

Unanticipated problems and adverse events must be reported to this office in accordance with the UT Health Science Center at Tyler/UT Health East Texas Human Research Protections Program (HRPP) Standard Operating Procedures.

The UT Health Science Center/UT Health East Texas Institutional Review Board is organized, operates, and is registered with the United States Office for Human Research Protections according to the regulations codified in the United States Code of Federal Regulations at 45 CFR 46 and 21 CFR 56. The UT Health Science Center/UT Health East Texas Institutional Review Board operates under Federal Wide Assurance Numbers: 00003494 and 00006044

Any complaints or issues of non-compliance must be immediately reported to this office. If you have any questions or comments about this correspondence, please contact the IRB Office at 903-877-7632 or irb@uthct.edu

Sincerely,

Paul Latta, DDS
Chairman, Institutional Review Board

Appendix-7
FDA Exempt

U.S. FOOD & DRUG ADMINISTRATION

IND 161831

EXEMPT IND

Musharaf Mohiuddin, MBBS, MPH
Research and Teaching Assistant
The University of Texas at Tyler
701 Olympic Plaza Circle
Tyler, TX 75701

Dear Dr. Mohiuddin:[1]

Please refer to your investigational new drug application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act for Carbogen (95% Oxygen, 5% Carbon Dioxide) gas.

After reviewing the information contained in your submission, we have concluded that your study, protocol entitled "*Transcranial Doppler (TCD) assessment of cerebral vasomotor reactivity (CVMR) using CO2 stimulus as an initial measure of neurological impact among post-covid-19 neurological syndrome (PCNS)*", meets all of the requirements for exemption from the IND regulations. Therefore, an IND is not required to conduct your investigation and any future submissions should not be submitted to this exempted IND. In accordance with 21 CFR 312.2(b)(4) of the regulations, FDA will not accept your application.

The IND regulations [21 CFR 312.2(b)] state that the clinical investigation of a drug product, including a biological product, that is lawfully marketed in the United States, is exempt from the requirements for an IND if all of the following apply:

(1) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use, nor intended to be used to support any other significant change in the labeling for the drug.

(2) The investigation is not intended to support a significant change in the advertising for a prescription drug product.

(3) The investigation does not involve a change in route of administration, dosage level, or patient population, or other factor that significantly increases the risks (or decreases the acceptability of risks) associated with use of the drug product.

[1] We update guidances periodically. For the most recent version of a guidance, check the FDA Guidance Documents Database https://www.fda.gov/RegulatoryInformation/Guidances/default.htm.

(4) The investigation is conducted in compliance with the requirements for institutional review (21 CFR Part 56) and informed consent (21 CFR Part 50).

(5) The investigation is conducted in compliance with the requirements of 21 CFR 312.7, i.e., the drug may not be represented as safe or effective, nor may it be commercially distributed, for the purposes for which it is under investigation.

In addition, 21 CFR 312.2(b)(5) exempts from the IND requirements a clinical investigation that involves use of a placebo if the investigation does not otherwise require submission of an IND.

We remind you that exemption from the requirements for an IND does not in any way exempt you from complying with the requirements for informed consent under 21 CFR 50.20 or from initial and continuing Institutional Review Board review under 21 CFR Part 56. You are also responsible for complying with the applicable provisions of sections 402(i) and 402(j) of the Public Health Service Act (PHS Act) (42 U.S.C. §§ 282(i) and (j)), which was amended by Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) (Public Law No. 110-85, 121 Stat. 904).

Title VIII of FDAAA amended the PHS Act by adding new section 402(j) (42 USC § 282(j)), which expanded the current database known as ClinicalTrials.gov to include mandatory registration and reporting of results for applicable clinical trials of human drugs (including biological products) and devices. Please note that, if in the future you submit an application under sections 505, 515, or 520(m) of the FDCA (21 USC §§ 355, 360(e), or 360(j)(m)), or under section 351 of the PHS Act (21 U.S.C. § 262), or you submit a report under section 510(k) of the FDCA (21 USC § 360(k)), the application or submission must be accompanied by a certification that all applicable requirements of section 402(j) of the PHS Act (42 USC § 282(j)) have been met. Where available, such certification must include the appropriate National Clinical Trial (NCT) control numbers (42 USC § 282(j)(5)(B)). Additional information regarding the certification is available at FDA.gov.[2] Additional information regarding Title VIII of FDAAA is available at NIH.gov.[3] Additional information on registering your clinical trial(s) is available at the Protocol Registration System website.[4]

For additional information about IND regulations, you can check our web site.[5]

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[2] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/form-fda-3674-certifications-accompany-drug-biological-product-and-device-applicationssubmissions
[3] http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-014.html
[4] http://prsinfo.clinicaltrials.gov/
[5] http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm

U.S. Food and Drug Administration
Silver Spring, MD 20993
www.fda.gov

If you have any questions, contact Tina Chhabra, Regulatory Project Manager via email at Tina.Chhabra@fda.hhs.gov.

Sincerely,

{See appended electronic signature page}

Nick Kozauer, MD
Director
Division of Neurology 2
Office of Neuroscience
Center for Drug Evaluation and Research

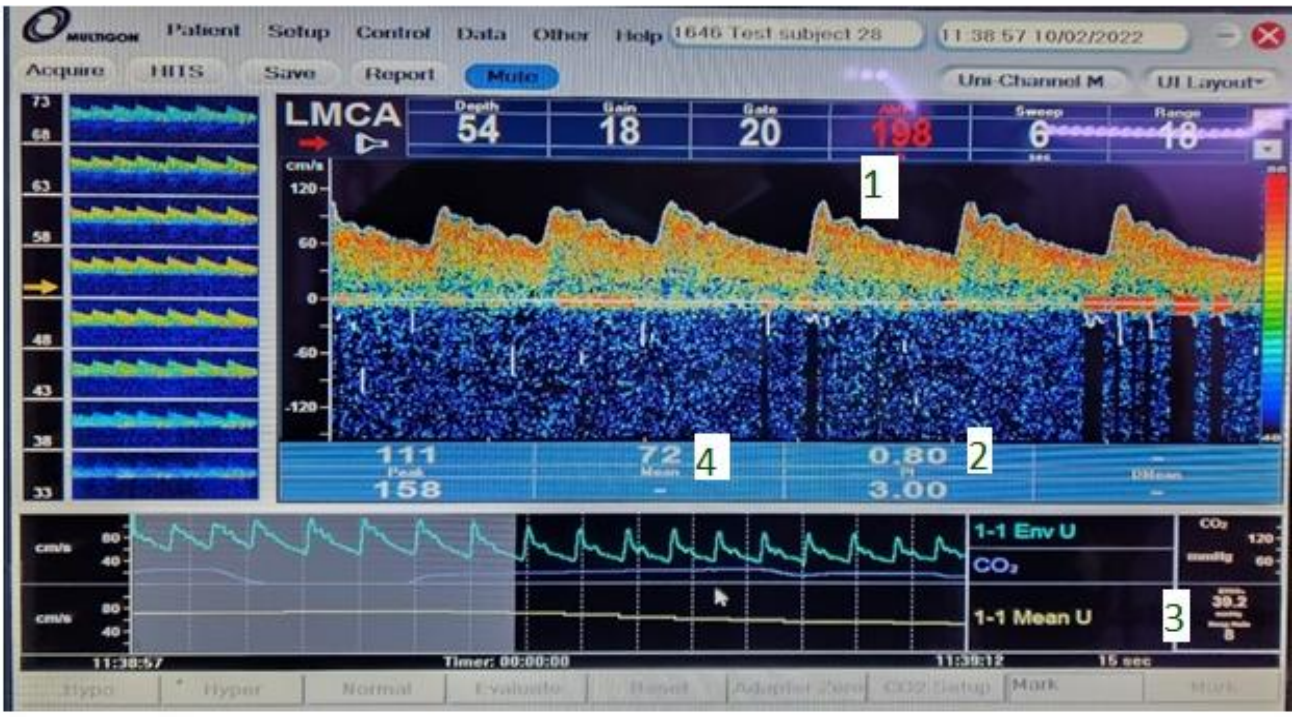

Figure 1: TCD Screen snapshot. 1. A real-time spectral display of the left middle cerebral artery with mean flow velocities (MFV) and Gosling pulsatility indices. 2. End-tidal CO_2. 3. Respiration rate. 4. Blood velocity at 10cm/sec

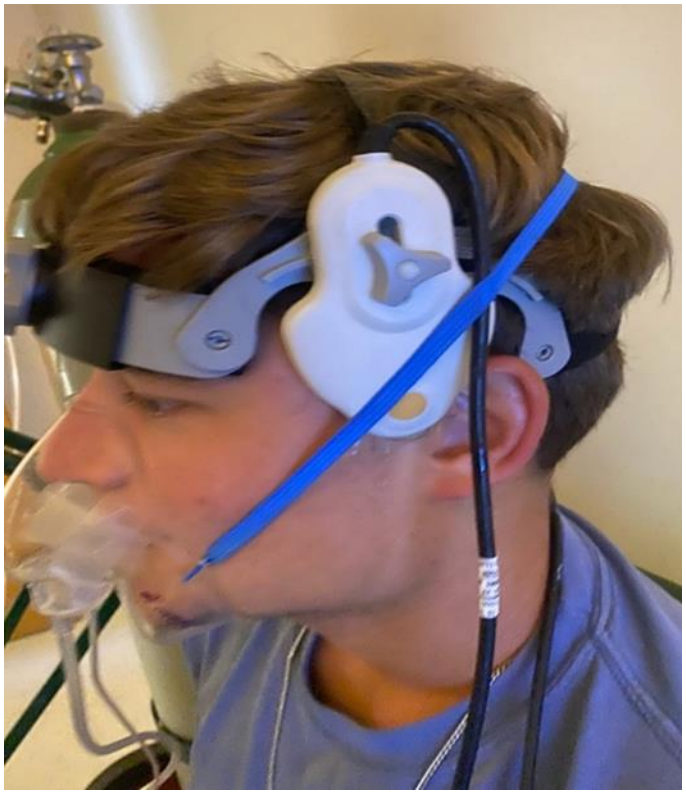

Figure 2. TCD hydraulic Monitoring headband in place with Dual-Port disposable face mask.

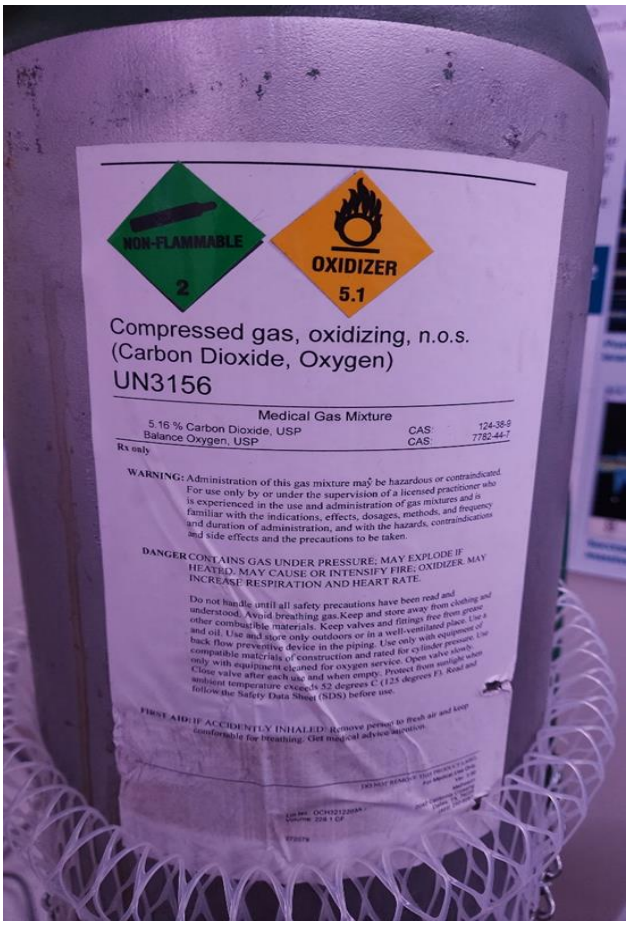

Figure 3: Carbogen Tank