PROTEOMIC BIOMARKERS, NEUROQUANT® DATA, AND SYMPTOMS OF BRAIN NEURONAL INJURY IN ADULTS WITH SUBJECTIVE COGNITIVE IMPAIRMENT

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ABSTRACT

Acquired brain neuronal injury is one of the most significant healthcare problems nationally and globally. Acquired brain neuronal injury results from hypoxemia, biotoxin or ribotoxin exposure, or pressure shifts in the brain neuronal architecture and is responsible for 45% of 42 million dementia cases globally. Biological changes in the brain start decades before cognitive impairment is identified and may be delayed or reversible.

In a retrospective study using secondary data analysis, we examined the interrelationships among proteomic biomarkers transforming growth factor-beta1 (TGFβ1), vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and complement C4a, and NeuroQuant® data with symptoms of subjective cognitive impairment (SCI) in 30 to 59 years old adults.

As an exploratory analysis not previously investigated for NeuroQuant® data, 195 female and 55 males NeuroQuant® data sets were extracted. There were multiple correlations among upregulated C4a, TGFβ1, and VEGF and the intracranial volume (IVC) to the forebrain, cortical grey, ventricles, hippocampus, pallidum, thalamus, caudate, and amygdala. Adults with self-reported poor memory demonstrated volumetric atrophy to the hippocampus, cortical grey, and

cerebellum. There were insufficient aged-matched controls to examine the relationship between sociodemographic data, proteomic biomarkers, and NeuroQuant® data in adults.

Peripheral biomarkers C4a, TGFβ1, and VEGF are potential indicators of brain parenchymal changes in adults with SCI. Our data suggest pathologies associated with neuronal injury are present in the brain and plasma in adults with SCI. Early screening for acquired brain neuronal injury is essential to allow prompt interventions and prevent SCI progression to mild cognitive impairment (MCI). Routine screening for brain neuronal injury should begin early in primary care and not solely focus on poor memory as an indicator of cognitive impairment. SCI should be investigated further with proteomics, NeuroQuant® examination, and lifestyle alterations.

Future research should include examining chromosomal sex differences in brain neuronal injury, the impact of the female brain's perimenopausal transition to develop a comprehensive understanding of the dynamic metabolic aging process, potential interventions, and possible window of prevention for brain neuronal injury. Additionally, scholarship should investigate molecular hypometabolism and aged-matched controls for NeuroQuant® data, age, and gender.

DEDICATION

To over 1692 sun rises, and sun sets that will never return,

To my parents for their love and support,

To my husband, Joe, and children, Grace and Joe, for always being by my side,

encouraging me every step of the way,

And

To my alma mater, Hoya Saxa!

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Chapter I

Description of the Problem

Vascular pathology is recognized as a principal insult in an acquired brain neuronal injury (Chan, Pole, Keightley, Mann & Colantonio, 2016). An acquired brain neuronal injury results from oxygen restriction to the brain, exposure to biotoxins and endotoxins, or pressure shifts that cause alterations in brain neuronal architecture (Alzheimer's Association, 2018). Loss of brain function due to vascular pathology accounts for at least 25% of the approximately 17 million strokes in adults reported annually worldwide (Chen, et al., 1999; Hainsworth, et al. 2017). Silent neurovascular dysfunction is estimated to contribute up to 45% of the approximately 42 million dementia cases reported globally each year and is a significant cause of long-lasting disability (Chen, 1999, World Health Organization [WHO], 2017; Alzheimer's Association, 2018). Focal and diffuse degeneration of the brain underlies disruptions in memory and cognition, as well as personality changes. Ultimately, a vascular impairment may lead to brain failure (Wardlaw, et al., 2017).

Research demonstrates these biological changes in the brain start decades before cognitive impairment becomes observable (Beason-Held et al, 2013). Cognitive decline associated with brain degeneration may be delayed or reversible (Bredesen, 2016; Bredesen et al., 2016) although the reported research is in its infancy. There are no current published studies that examined the associations among brain neuronal injury, symptoms, NeuroQuant® data, and proteomic biomarkers Thus, it is clinically important to examine these associations to elucidate the mechanisms of vascular dysfunction in neurodegeneration.

Blood-Brain Barrier

The blood-brain barrier (BBB) is a dynamic functional interface between the peripheral blood circulation and the central nervous system (CNS), which allows the transport of nutrients, essential amino acids, ions, and other components between the peripheral circulation and the brain. At the same time, the BBB inhibits pathogens and toxic compounds from entering the brain (Grammas, Martinez, & Miller, 2011; Daneman & Prat, 2015). The BBB primary functions are to maintain homeostasis of the brain and to protect the brain from potentially endogenous insult and xenobiotics to maintain optimal neuronal activity (Sivandzade & Cucullo, 2018). The BBB is formed from the brain endothelial cells (Erdo, Denes, & DeLange, 2016). The junctions between these endothelial cells are tightly connected through the adherens junction (AJ) proteins such as cadherins and tight junctional proteins such as occludin and claudins (Erdo, Denes, & DeLange, 2016). Astrocytes, microglia, and pericytes are essential for BBB normal function and also for the phenotype of brain endothelial cells for their contribution to the formation and maintenance, selectivity, and specificity of the BBB (Broux, Gowing, & Prat, 2015). Chowen et al. (2016) described the essential role of astrocytes in the regulation of available nutrients, such as glucose, and peripheral hormones, including leptin, ghrelin, and glucagon-like-peptide-1 (GLP-1). Their anatomical position, which is in close proximity to the blood vessels and neurons, allows the astrocytes to function as important metabolic sensors.

Hypothalamic astrocytes are involved in the control of energy homeostasis by transporting glucose into the CNS. The CNS glucose is stored in the form of glycogen, which is mobilized to release lactate to the neurons when glucose is not abundant, for example, during periods of ischemia (Argente-Arizón et al., 2015; Chowen et al., 2016). The physiological processes that

glycogen supports, such as learning and memory, promote an inclusive and vital role in supporting metabolic brain functions and are crucial in maintaining brain homeostasis.

Several neurotransmitters, biochemical mediators, pro-inflammatory cytokines, and epigenetic changes play significant roles in the molecular mechanisms of brain neuronal injury (Ladak, Enam, & Ibrahim, 2019). This pro-inflammatory environment facilitates the activation and the influx of immune cells into the white matter of the brain parenchyma determining the progression of injury including excitotoxicity and neuronal loss (Varatharaj & Galea, 2017). Several studies reported that chronic neuronal injury leads to the onset of cerebrovascular and neurodegenerative disorders such as Alzheimer's disease, chronic traumatic encephalopathy, and epilepsy, as well as, other long-term problems, including the loss of executive function, inappropriate social behavior, and cognitive disabilities (Bredesen, 2016; Bredesen, Amos, Canick, Ackerley, Raji, Fiala, Ahdidanet, 2016; Shoemaker, 2010; Grattan, et al. 1998; Block, et al. 2012; de la Monte, 2017; Schulingkamp, Pagano, Hung, and Raffa, 2000; Watson and Craft, 2003).

Disruption of the BBB associated with neuronal injury promotes the activation of the coagulation cascade, resulting in intravascular blood clotting and distal ischemia (Dejana, Tournier-Lasserve, Weinstein, 2009; Ahn, Baker, Norris, Strickland, 2019). The resulting hyperpermeability ultimately induces neuroinflammation by promoting the entry of transforming growth factor-beta1 (TGFβ1), vascular endothelial growth factor (VEGF), and matrix metalloproteinase-9 (MMP-9), which become abnormally elevated in the brain further contributing to BBB impairment and the loss of barrier integrity (Muradashvili & Lominadze, 2013). The BBB damage will eventually facilitate the development of cerebral interstitial edema (Price, Wilson, & Grant, 2015). Moreover, the upregulation of VEGF, which is a significant

regulator of endothelial cell proliferation, angiogenesis, and vascular permeability, as well as, the downregulation of claudin-5 expression, was correlated with BBB dysfunction (Suzuki, Nagai, & Umemura, 2016; Nag, Manias, Eubanks, & Stewart, 2019).

As VEGF levels in the body rise with vascular wall damage, TGFβ1, a pleiotropic cytokine with a pivotal role in cell proliferation and differentiation, is released into the peripheral circulation. VEGF-mediated apoptosis is required for TGFβ1 angiogenesis (Konig, Kogel, Rami, & Prehn, 2005; Lindholm, Castren, Kiefer, Zafra, & Thoenen, 1992; Ferrari, Cook, Terushkin, Pintucci, & Paolo, 2009). TGFβ1 has been shown to protect cultured neurons from hypoxic (Prehn, Backhauss, & Krieglstein, 1993; Ferrari, Cook, Terushkin, Pintucci, & Paolo, 2009), excitotoxic (Prehn & Krieglstein, 1994; Ferrari, Cook, Terushkin, Pintucci, & Paolo, 2009), apoptotic (Prehn, Backhauss, & Krieglstein, 1993; Ferrari, Cook, Terushkin, Pintucci, & Paolo, 2009), and metabolic insults (Krieglstein, Suter-Crazzolara, Fisher & Unsicker, 1995; Ferrari, Cook, Terushkin, Pintucci, & Paolo, 2009). In addition, TGFβ1 plays a role in maintaining BBB integrity through stabilizing endothelial cells (Ferrari, Cook, Terushkin, Pintucci, & Paolo, 2009; Shen, et al., 2011).

Disruption of the BBB leads to the white matter damage that is observed on brain magnetic resonance imagery (MRI) and termed white matter hyperintensity (WMH). Debette and Markus (2010) suggested that WMH is a disruption of the neural network, which predisposes the individual to an accelerated decline in cognitive function and is associated with functional decline, gait disturbance, and depression. Thus, WMH may be interpreted clinically as a surrogate biomarker for cerebral small vessel disease (cSVD) (Chutinet & Rost, 2015). The reduced blood flow leads to hypoxia, alters mechanisms of cerebral autoregulation, and promotes

the increase in pro-inflammatory biomarkers, which leads to further degradation of both the BBB and the brain parenchyma.

As this degradation progresses, increased axonal loss further potentiates demyelination and atrophy of the overlying cerebral cortex. However, the literature shows that there are no clinical guidelines regarding WMH diagnosis. In addition, Debette and Markus (2010) identify the need for computer-based algorithms given the increased interest in brain research and in the context of clinical studies, an automated approach to detection of WMH is desirable.

The BBB degradation is further potentiated by the prolonged excitation of the amino acid glutamate, in response to neuronal injury (McKee and Daneshvar, 2015). This cause-and-effect function of glutamate results in neuronal dysfunction, neuroinflammation, and underlying metabolic cellular dysfunction. Warburg, Wind, & Negelin (1927) identified that under aerobic conditions, oxidative phosphorylation is blocked, forcing cells to generate adenosine triphosphate (ATP) by converting glucose to lactate via glycolysis. Although this is a highly inefficient use of glucose, it does provide building blocks needed for cell proliferation, generating only 2 ATP molecules per glucose instead of 36–38 ATP molecules per glucose with complete oxidation. Yet under aerobic conditions, metabolism is switched from oxidative phosphorylation to aerobic glycolysis, and as recently explained, glutaminolysis, in which glucose is fermented to lactate despite the presence of oxygen (Warburg, Wind, & Negelein, 1927; Wang, Marquardt, & Foker, 1976; Yang, Venneti, & Nagrath, 2017). The tricarboxylic acid cycle (TCA cycle) is switched from glucose to glutamate to glutamine (DeBerardinis, et al. (2007).

The abundance of glutamate further compromises the voltage-dependent anion-selective channel (VDAC) responsible for the transport of metabolites and regulation of mitochondrial

function. Research into the physiology and pathophysiology of VDACs and mitochondrial translocases, which allows for the movement of proteins through the outer-wall membrane into the intermembrane space of the mitochondrion. This movement across the mitochondrial membrane has become a focus in neoplastic and neurodegenerative diseases. The VDAC is recognized as a gatekeeper for normal mitochondrial function and as a key factor in both cytoprotection, and a mediator of mitochondria-induced apoptosis (Camara, Zhou, Wen, Tajkorshid, & Kwok, 2017).

Ishikawa (2013) described how hypoperfusion of the CNS down-regulates both glutamate and glutamine synthetase leading to depletion of ATP. Furthermore, the excitotoxicity results in a negative feedback look of oxidative stress that impacts mitochondrial dynamics, further increasing the excitotoxicity. Glutamine synthesis consumes ATP as a protective mechanism against excitotoxicity. This cascade of events illustrates the importance of maintaining homeostasis of glutamate metabolism to prevent the deleterious effects of cSVD.

Pathological changes at molecular and cellular levels associated with neurodegenerative diseases precede the clinical onset by several years underscoring a critical clinical need to initiate interventions before the onset of neurological symptoms. If left untreated, cognitive impairment will progress to neurodegenerative pathology (Chen, et al., 1999). Increasing evidence shows that neuronal death and cognitive deficit predate the neurological diagnosis by up to twenty years (Villemagne et al., 2013). The timing of symptom progression from mild to moderate severity varies. Initially, the patient compensates for early changes in symptom severity allowing normal functioning, which is termed subjective cognitive impairment (SCI). Mild cognitive impairment (MCI) follows SCI, and this stage is reported to have a high probability of progression to neuronal injury (Kantarci et al, 2008). As the severity of brain neuronal injury increases, the

individual can no longer compensate for this injury and begins to exhibit subtle cognitive changes. As the progressive brain injury becomes more significant, the observable cognitive decline becomes more frequent with increased memory loss or confusion. The advent of memory or cognitive decline observable by others denotes the progression from SCI to MCI (Alzheimer's Association, 2018).

Cognitive impairment is often multifactorial with outcomes of short and long-term memory dysfunction, thinking, and decision-making. Frequent causes of cognitive impairment include metabolic imbalances such as the insulin resistance found in type II diabetes mellitus and obesity, neurodegenerative diseases such as Alzheimer's or dementia, systemic infections, as well as, exposure to environmental toxins such as mycotoxins, actinomyces, endotoxins, inflammagens, heavy metals (Grattan, et al. 1998; Schulingkamp, Pagano, Hung, & Raffa, 2000; Watson & Craft, 2003; Shoemaker, 2010; Block, et al. 2012; Bredesen, 2016; Bredesen et al., 2016; de la Monte, 2017). The WHO (2017) estimates that 12% of adults aged 45 years or older have some symptoms of SCI. Of great concern is the finding that 85% of this population rarely discusses this decline with their primary care provider. Research focus has transitioned from examining subjects diagnosed with an advanced neuronal injury, e.g., Alzheimer's Disease or dementia, to clinically healthy subjects with self-reported concerns about changes in their cognition (Russo, Murrough, Han, Charney & Nestler, 2012).

Research is focusing currently on the etiology and treatment of neuroinflammation and cerebral neuronal injury (Furman, et al. 2019). Therapeutic approaches to these complex inflammatory processes in the brain remain limited, and thus, there is a critical need for translational research. Macro- and microvascular pathology leading to brain endothelial dysfunction are central to the pathogenesis and clinical manifestations of SCI (Poggesi, Pasi,

Pescini, Pantoni, & Inzitari, 2016). Only recently has the technology become sensitive enough to quantify SCI. Conducting clinical research in this population is challenging because vascular brain dysfunction is difficult to diagnose and monitor due to the difficulty in obtaining tissue samples from the brain and limited non-invasive technology for capillary level assessment of the nascent injury. Recent specialized post-processing radiologic brain imaging termed NeuroQuant® has given researchers a sensitive tool to identify, quantify, and monitor areas of potential brain pathology (England, Gillis, & Hampstead, 2014). Presenting symptoms of a structural brain abnormality depend on the specific type, extent, and duration of this brain abnormality and include a disturbed thought process, mood disturbance, behavior, and memory changes.

The few pharmaceutical agents that are available to slow the progression of neurovascular dysfunction and neurodegeneration have not demonstrated efficacy. In fact, Cummings, Morstorf, and Zhong (2014) examined 413 Alzheimer's potential treatment which included 244 investigational compounds between 2002 and 2012. Of the 244 compounds, only one was approved. Alzheimer's disease drug candidates have the highest failures rates of any disease area with 99.6%, compared with 81% for cancer. For the compounds approved by the Food and Drug Administration, cholinesterase inhibitors and memantine have no impact on the reversal of cognitive impairment (Cui, Sun, Wang, Zhang, & Xing, 2019). The WHO (2016) has classified CNS neuronal injury as a public health priority that requires studies designed to test strategies for early identification of neuronal injury and efficacy of interventions.

The WHO (2020) identified specific research goals to identify early pathologic changes associated with a neuronal injury. For example, abnormal accumulation of amyloid and tau protein begins 10-20 years prior to the onset of cognitive dysfunction (Beason-Held, et al., 2013). In addition, cerebral cortex and hippocampal atrophic changes with lateral ventricle

enlargement are often present in those diagnosed with MCI (Beason-Held, et al., 2013). Research needs include the development of a clinical tool that identifies consistently those individuals with biomarkers for SCI in the early stages of pathologic changes associated with brain neuronal injury. Thus, this project is designed to examine the relationship among proteomic biomarkers, NeuroQuant® Data, and symptoms as clinical indicators of neuronal injury in adults with subjective cognitive impairment.

Background and Significance of the Problem

Vascular dysfunction is a significant health challenge and an early indicator of many neurodegenerative diseases, including diseases like Alzheimer's Disease and dementia (Strickland, 2018; Horsburgh, et al., 2018; Chen, et al., 1999; Hainsworth, et al. 2017). Vascular dysfunction seen in cSVD occurs in diverse types of cognitive impairment.

Cerebral Small Vessel Disease

Mok and Kim (2015) described (always use past tense for previous research pubs) cSVD as a group of pathologic processes with various etiologies that affect the small arteries, arterioles, venules, and capillaries of the brain. Although the pathogenesis of cSVD is unclear, several studies have suggested that systemic inflammation is a key contributor (Wardlaw, et al., 2013; Furman, et al., 2019). Inflammation is the immune system's defense mechanism to respond to stressors, including ischemia, injury, and infection. These salutary processes act through a complex, multi-step process beginning with microglial activation, cytokine/chemokine release, and macrophage/neutrophil infiltration direct to the affected tissue, followed by elimination of the precipitating stressor, and repair of the tissue injury (Chen, et al., 1999; Wang et al., 2007; Jordan et al., 2008; Furman, et al., 2019). Inflammation is involved in the interactions between cell surface receptors, the cellular extracellular matrix, and proinflammatory mediators (Keane &

Strieter, 2000). Severe chronic inflammation is well-described as having detrimental effects on the nervous system and contributes to the progression of chronic diseases such as Alzheimer's Disease, Parkinson's disease, and epilepsy (Horsburgh, et al., 2018; Chen, et al., 1999).

Over the last few decades, evidence has shown the prevalence of and clinical significance of cSVD in ischemic and hemorrhagic stroke, microbleeds, brain atrophy, chronic hypoperfusion, and increased BBB permeability (Wallin, et al., 2018). Strickland (2018) has posited the vascular hypothesis of neuronal injury as phenotypically altered micro-vessels that may affect the local circulation of the surrounding tissue and lead to tissue hypoxia and metabolic alterations. Subsequently, local hypoxia in the brain results in SCI and fatigue, which are the most prominent symptoms reported in diseases with chronic inflammation and immune dysfunction (Poggesi, et al., 2016).

One of the hallmarks of neurovascular or systemic injury-related inflammation is an increase in vascular permeability, which is driven frequently by excess nitric oxide, reactive oxygen species (ROS), local release of lactate, and other mediators (Ahn, Baker, Norris, & Strickland, 2019; Dejana, Tournier-Lasserve, & Weinstein, 2009). The increased neurovascular permeability allows plasma components and inflammatory cells to migrate outside of the bloodstream. These cells form a provisional matrix with plasma proteins, including MMP-9, that create a scaffolding for the migration of infiltrating leukocytes to the subendothelial space to initiate and sustain the inflammatory response.

Ahn, Baker, Norris, and Strickland (2019) identified that the barrier function of blood vessels is essential for homeostasis maintained via tight junctions. Further, Dejana et al. (2009) discussed how tight junctions are organized through claudins, occludins, and junction adhesion molecules. The extracellular complexes and intracellular links to the cytoskeleton maintain and

regulate the vascular barrier (Ahn, Baker, Norris, & Strickland, 2019; Dejana, Orsenigo, & Lampugnani, 2008). The dynamic, local control of vascular permeability enables macromolecular transport, immune surveillance, and the rapid generation of a fibrin-rich provisional matrix via the deposition of serum proteins (Strickland, 2018; Wu, 2005).

Chronic Cerebral Hypoperfusion

There is substantial evidence that cSVD results in chronic cerebral hypoperfusion due to the altered caliber of the cerebral small vessels resulting in incomplete ischemia of the white matter. The hypoperfusion is accompanied by inflammation, diffuse rarefaction of myelin sheaths, axonal disruption, and astrocyte gliosis (Duncombe, et al., 2017). The effects of chronic cerebral hypoperfusion on cognitive impairment have not been fully elucidated. Although acute cerebral hypoperfusion causes cerebral infarction through necrosis of neurons, chronic cerebral hypoperfusion is expected to cause neuronal apoptosis (Shama & Hassan 2010; Broughton, Reutens, & Sobey, 2009). Erecinska and Silver (1989) discussed the importance of a consistent, optimal supply of blood and glucose for appropriate mitochondrial ATP production in the maintenance of neuronal activity and structural brain integrity. In the last decade, research evidence has suggested that chronic cerebral hypoperfusion induces neurodegeneration via neuronal energy depletion and the generation of ROS and proinflammatory cytokines from activated microglial cells (Mittal, Siddiqui, Tran, Reddy, & Malik, 2014; Popa-Wagner, Mitran, Sivanesan, Chang, & Buga (2013). Strickland (2018) proposed cSVD as a possible cause for chronic cerebral hypoperfusion. Arba et al. (2017) identified white matter hyperintensities observed on MRI to be caused by cSVD. In addition, normal aging has been associated with cSVD with a 20% decrease in cerebral blood flow at the age of 60 years as compared to the age of 20 years (Heo et al. 2010).

Blood brain barrier disruption is an integral feature of numerous neurological diseases. A central question in these diseases is the extent to which inflammatory immune cells contribute to the disruption of the BBB and ensuing CNS vascular permeability. Research is needed to elucidate this contribution to develop therapeutic approaches to treat the pathology associated with BBB disruption in neurological diseases.

Vascular Endothelial Growth Factor and CNS Vascular Permeability

Cerebral capillary hypoperfusion occurs when CD8 T cells cause a disruption of the BBB tight junction proteins and increase in the CNS vascular permeability in the absence of neutrophil support and other perforin cells. Clinical observations and current experimental model systems used to address the contribution of inflammatory cells in BBB disruption support a potential role for VEGF, a cytokine that has an instrumental role in vascular development and angiogenesis (Nag, Takahaski, & Kilty, 1997; Nag, Manias, Eubanks, & Stewart, 2019). As Weis and Cheresh (2005) described, VEGF was originally defined as a "vascular permeability factor" and is 50,000-fold more potent than histamine in promoting vascular permeability as seen in in vitro assays. This demonstrates the potency of this cytokine to promote the deregulation of the vasculature. Vascular endothelial growth factor - mediated permeability occurs through modification of endothelial cells to develop a) vesiculo-vacuolar organelles; b) increased caveolae formation; c) fenestrations in membranes; and d) alteration of tight junctions (Bates, 2010; Apte, Chen & Ferrara, 2019). Apte, Chen, and Ferrara (2019) identified the prevailing hypothesis that VEGF effects on vascular endothelial cells result in local edema, hemorrhage, and tissue damage. The specific interactions between inflammatory cells that result in VEGF-mediated vascular permeability remain to be elucidated. Assessment of the importance of VEGF

in the CNS is further complicated by the dual role of this cytokine in angiogenesis and development, as well as, in mediating vascular permeability.

Matrix Metalloproteinase and CNS Vascular Permeability

Cerebral ischemia and reperfusion are two pathological conditions that lead to ROS and BBB breakdown. Current research suggests that MMP-9 is activated by ROS produced during central ischemia and reperfusion. The up-regulation of MMP-9 is one of the main actions responsible for degrading the extracellular matrix (ECM) proteins of the cerebral vascular basal membrane and the tight junctions between cerebral endothelial cells. This degradation leads to the disruption of BBB integrity, which in turn has significantly negative consequences on the CNS, resulting in brain edema, infiltration of inflammatory cells, secondary brain damage, and poor neurological outcomes (Rosell et al. 2006; Zlokovic, 2006). A lack of oxygen and nutrient delivery due to a decrease in blood flow to part of the brain damages the tissues. Brain neuronal injury activates MMP-9, disrupting the BBB, and contributing to neuroinflammation and neurodegeneration (Abdul et al., 2017).

Trans Growth Factor Beta - 1 and CNS Vascular Permeability

Trans Growth Factor beta-1expression is confined primarily to the meninges and choroid plexus. This cytokine is implicated in the control of cell growth, differentiation, inflammation, and apoptosis. TGFβ1 is a key factor in BBB disruption, hemorrhage, neuroinflammation and cell death in multiple neurological diseases, such as AD, amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) (Chen, et al., 1999; Manaebko, Lekic, Barnhart, Hartman, & Zhang, 2014; McMillin et al, 2015). The up regulation of TGFβ1 has also been identified in diverse pathological conditions such as multiple sclerosis, stroke, AD, tumors, and trauma (Kim et al., 2017).

Complement 4a and CNS Vascular Permeability

Complement 4a (C4a) is a small protein released during complement component C4 activation by the classical and lectin complement pathways, which are essential constituents of innate immune surveillance (Roumenina, Rayes, Frimat, & Bacchi, 2017). Further injury accrues via auto-activation of C4a production by mannose binding lectin associated serine proteases (MASP2) (Wallis, 2009). The host defense relies on a tight interplay and extensive crosstalk between innate immune pathways such as the complement system, the contact and coagulation cascades, and cellular components, including platelets and endothelial cells (ECs) (Wang, Ricklin, & Lambris, 2017). Although typically conferring protection from microbial intruders and accumulating debris, host defense systems can contribute to various thrombo-inflammatory, acute-phase, and age-related disorders if inappropriately triggered (Ricklin, Reis & Lambris, 2016). C4a plays a vital role in these processes by sensing pathogen- and damage-associated molecular patterns and initiating a cascade that facilitates the elimination of invading cells via phagocytosis and lytic damage and releasing inflammatory mediators to propagate danger signaling (Roumenina, Rayes, Frimat, & Bacchi, 2017).

Visual Contrast Sensitivity and Neuronal Injury

Visual contrast sensitivity (VCS) describes the ability to distinguish visual images of variable light-dark contrast. Visual contrast sensitivity is dependent upon both the optical components of the eye, which focus images on the retina, neural rim of optic nerve head and visual pathways of the optic radiation in the CNS that transmit and integrate neural signals in the brain. Numerous studies have shown visual system dysfunction in patients with MCI (Albers, et al., 2015). Bublak et al. (2011) identified impaired visual contrast sensitivity as a non-specific pathologic biomarker of cognitive impairment. Visual contrast sensitivity testing has been used

to document neurologic injury in illnesses caused by exposure to biotoxins that are distinct markers of neuronal injury independent of memory deficits (Grattan et al., 1998; Shoemaker, 2001).

It has become increasingly important to find biomarkers of neuroinflammation that can be used to track neurodegeneration and progression. This project is designed to specifically elucidate the mechanisms underlying BBB dysfunction and the inflammatory response associated with a neuronal injury in adults with SCI. The project will examine symptom clusters, serum biomarkers, and brain MRI with NeuroQuant® diagnostic data in adults with neuronal injury and SCI. Most neurodegenerative brain disorder research to date has examined tau and phosphorylated tau and their specific roles in the pathogenesis of brain atrophy. This project will test a novel hypothesis regarding the etiology of brain neuronal injury and related SCI. Two hypotheses will be tested in this study: 1) There will be a statistically significant positive increase in the levels of proteomic plasma biomarkers in the presence of SCI; 2) There will be statistically significant positive correlation between SCI and brain MRI with NeuroQuant® data. To our knowledge, this is the first project designed to examine brain neuronal injury, symptoms, and proteomic biomarkers in patients ≤ 60 years of age.

Research Question

The research question for this project is "What are the proteomic biomarkers, brain MRI NeuroQuant® data, and symptoms associated with neuronal injury in adults with SCI? Thus, this project is designed to examine the relationship among proteomic biomarkers, NeuroQuant® Data, and symptoms as clinical indicators of neuronal injury in adults with subjective cognitive impairment." The question was formulated utilizing the population - intervention - comparison - outcome - time (PICOT) framework (Aslam and Emmanuel, 2010).

Organizational Needs Assessment

The PI obtained an organizational-wide commitment to support this scholarly project from stakeholders including the medical clinic leadership, the primary care provider, and staff members. The advantages of a retrospective study design include the ability to identify factors associated with SCI and generate future hypotheses to be studied. Potential barriers to this study included inconsistent data collection by the study site staff and missing data.

Theoretical Framework

Evidence-based practice (EBP) is a problem-solving approach to clinical decision-making. As described by White, Dudley, and Terhaar (2016), the increasing complexity of the healthcare delivery system presents provider challenges. The tremendous growth in new clinical and basic scientific data has outpaced clinicians' abilities to incorporate new evidence into data-based clinical practice. Incorporating the EBP approach requires the synthesis of the best available research with clinical expertise and patient preferences to promote individualized care leading to appropriate patient outcomes (Dogherty, Harrison, Graham, Vandyk, & Keeping-Burke). EBP is designed to increase the effectiveness and the efficacy of specific interventions while in parallel, decreasing costs and safety risks. The Johns Hopkins Nursing EBP (JHNEBP) model is one model available to guide the EBP process (Deraholt & Dang, 2011).

The JHNEBP model is a problem-solving method for clinical decision-making. The model uses the three-step procedure termed PET that includes practice questions, evidence, and translation (Deraholt & Dang, 2011). The JHNEBP is used to translate research to bedside care through the promotion of best practices quickly and appropriately incorporated into patient care practices.

The consequences of neuronal injury affect the foundation of an individual's autonomy and may lead to overwhelming disruption of family dynamics, loss of income or earning potential, and costly lifetime expenses. Long-term severe cognitive and behavioral sequelae of neuronal injury potentially affect interpersonal relationships, schoolwork, professional work, and personal safety. These negative impacts to an individual's cognitive and behavioral functions pose many challenges for medical and nursing management. Integrating the JHNEBP evidence-based practice approaches to care of the patient with neuronal injury is critical in optimizing an individual's long-term safety, recovery, and functional ability.

Definition of Terms

Brain MRI: Magnetic resonance imaging (MRI) is a diagnostic procedure that uses a combination of a large magnet, radio frequencies, and a computer to produce detailed images of the brain. Unlike x-rays or computed tomography (CT scans), MRI does not use ionizing radiation (MRI, 2019).

Complement 4a (C4a): An anaphylatoxin and mediator of the inflammatory process, C4a increases vascular permeability and the contraction of smooth muscle (National Institutes of Health, n.d.). It plays an important role in the body's natural ability to ward off infection and in the pathogenesis of infection and inflammation. Facilitating the phagocytosis of immune complexes, viral particles, and toxic cell debris, C4a is a known marker of chronic inflammation whether occurring primarily as part of the disease or as the body's response to the pathological effects of neurodegenerative diseases (Akiyama et. al, 2000).

Dementia: A chronic or progressive deterioration of cognitive function beyond what is expected from healthy aging. Includes subtypes vascular dementia, frontotemporal dementia, Lewy body

dementia, AD, subjective cognitive impairment (SCI), and mild cognitive impairment (MCI) (Schneider, Arvanitakis, Bang, & Bennett, 2007).

Matrix metalloproteinases (MMPs): A group of enzymes that are responsible for the degradation of the extracellular matrix proteins during growth and normal tissue turnover (Wang, Tan, Yu & Tan, 2014).

Mild cognitive impairment (MCI): Described as the clinical phase prior to the diagnosis of subjective cognitive impairment. Neuropsychological testing is abnormal, which demonstrates deficits in memory, organization, speaking, calculating, and other cognitive abilities. Activities of daily living are still performed independently (Francis et al. 2017).

NeuroQuant®: A computer software program that is used in conjunction with the brain MRI to measure regional brain structures at risk for injury and declining brain function (Brewer, 2009). The NQ provides a volumetric expression of the brain identifying the size, shape, and volume of the brain regions compared to age-matched controls.

Subjective cognitive impairment (SCI): Worsening cognition, which is noticeable to the individual, but s/he tests normal on neuropsychological testing. Even in this early stage, magnetic resonance imaging (MRI) may show some shrinkage of diverse brain regions (Kiuchi et al. 2014). SCI often spans decades before progression to mild cognitive impairment is noticed or observed.

Transforming growth factor-beta 1 (TGF-β1): A polypeptide cytokine that is secreted protein that controls cell growth, cell proliferation, cell differentiation, and apoptosis (Akdis et al., 2016).

Vascular endothelial-derived growth factor (VEGF): A vascular growth factor important in vasculogenesis and angiogenesis (Apte, Chen, & Ferrara, 2019). Identified, isolated, and cloned

25 years ago (Ferrara & Adamis, 2016), VEGF targets endothelial cells but has multiple effects on additional cell types. VEGF is essential for physiologic vascular homeostasis in diverse cells and tissues.

Chapter II

Introduction to Search Criteria

The literature search focused on clinical data, serum biomarkers, and NeuroQuant® data related to neuronal injury and SCI. Electronic databases searched were the Cumulative Index of Nursing and Allied Health Literature (CINAHL), PubMed, and OVID. Inclusion criteria were a) population aged 30 to 59 years; b) cerebral small vessel vascular dysfunction (cSVD); c) brain MRI with NeuroQuant® data; d) plasma levels of C4a, TGFβ1, VEGF, and serum levels of MMP-9; e) neuronal injury; i) articles published in the English language; and j) publication years 2009 – 2019 (most recent studies in which cSVD-related changes had been assessed using current MRI technologies). Exclusion criteria were a) pediatric or adolescent population; and b) articles not published in the English language. Search terms were combined as follows: 1) neuronal injury AND each of the following terms: fatigue, MCI, plasma C4a, serum MMP9, plasma TGFβ1, plasma VEGF, white-matter hyperintensities, NeuroQuant®; and 2) cSVD AND fatigue, MCI, fixed plasma versus serum C4a, serum MMP9, serum TGFβ1, serum VEGF, white-matter hyperintensities, NeuroQuant® data.

The PubMed database search yielded nine results with seven articles (i) a case-control study designed to examine the reliability and differences between NeuroQuant® and FreeSurfer data in structural brain MRI measurements (Ochs, Ross, Zannoni, Abildskov, Bigler & Alzheimer's disease Neuroimaging Initiative, 2015); (ii) a case-control study examining the relationship between memory indices and temporal lobe integrity utilizing NeuroQuant® neuroimaging tool (England, Gillis, & Hampstead, 2014); (iii) a case-control trial examining the utility of early identification of neuronal injury (Yu, Sun, Wolz, Stephenson, Brewer, Fox, & the

Coalition Against Major Diseases and the Alzheimer's Disease Neuroimaging Initiative, 2014); (iv) a consensus statement identifying vascular dysfunction as a prominent and early feature in prodromal neuronal injury (Sweeney, et al., 2019); (v) a systematic review on the current understandings of cSVD and recommendations for the diagnosis of neuronal injury (Kalaria, 2016); (vi) Alzheimer's disease: A matter of blood–brain barrier dysfunction? (Montagne, Zhao, & Zlokovic, 2017); and (vii) a randomized control trial examining multidomain interventions and their impact on cognition.

The Cumulated Index to Nursing and Allied Health Literature (CINAHL) database yielded two articles that met the inclusion criteria: (viii) a cross-sectional trial that examined innate immune system involvement in neuronal injury (Noz, Telgte, Wiegertjes, Joosten, Netea, de Leeuw, Riksen, 2018); and (ix) a systematic review of literature (Leandrou, Petroudi, Kyriacou, Reyes-Aldasoro, & Pattichis, 2018).

The Ovid database identified five articles: (x) a case-control study examining the difference between NeuroQuant® and cognitive testing data in the early stages of neuronal injury (Allison et al., 2019); (xi) the third generation cohort of the Framingham Heart Study investigated the association of VEGF and MRI biomarkers of brain aging in middle-aged adults (Raman, et al, 2019); (xii) a case-control study to identify plasma proteins associated with inflammation within the complement pathway in neuronal injury (Bennett et al., 2012); (xiii) a systematic review to determine which plasma proteins appear in both the blood and brains of dementia patients (Khan et al., 2016); and (xiv) a case-controlled study to identify hippocampal loss in Alzheimer's disease, mild cognitive impairment, and normal aging (Schuff et al, 2009).

Synthesis of Previous Research

Vascular Dysfunction

Sweeney et al. (2019) identified vascular dysfunction as not only a causal pathway to neuronal injury, but also as a contributor to brain failure. Evidence of cSVD was identified in 80% of patients diagnosed with Alzheimer's Disease, which supports the hypothesis that cerebrovascular dysfunction is prominent in neuronal injury.

Kalaria (2016) reviewed the current understanding of neuronal injury and presented clinical, neuropsychological, and pathological indicators of the progression from cognitive dysfunction to dementia. In one study, 42% of the participants diagnosed with Alzheimer's disease were also diagnosed with cSVD. The author discussed that although the definitive diagnosis of dementia is made at autopsy, appropriate sampling and essential neuropathological examination are necessary to rule out other pathological changes associated with different causes of cognitive impairment. Kalaria (2016) identified limitations of previous research including sampling bias, small sample size, use of non-standard or difficult-to-compare assessment instruments for clinical, neuropsychological, neuroimaging, and neuropathological evaluation. More sensitive neuroimaging testing needs to be used to identify disease earlier than is possible at present. This earlier identification would allow precise and timely preventive and treatment strategies of all-cause neuronal injury.

MRI with NeuroQuant® Analysis

England, Gillis, & Hampstead (2014) reported that volumetric MRI using NeuroQuant® was superior to neuropsychological tests for early diagnosis of neurodegenerative diseases such as Alzheimer's disease. Volumetric MRI is the most sensitive, noninvasive method to examine

brain soft-tissue changes, which occur early in the progression of neuronal injury. The authors determined that NeuroQuant® assessment can aid in the staging of clinical progression for MCI.

Leandrou, Petroudi, Kyriacou, Reyes-Aldasoro, & Pattichis (2018) identified hippocampal loss in patients with MCI. They also found that early stages of neuronal injury are significant indicators of temporal lobe atrophy. As the neuronal injury progresses to Alzheimer's disease, both the hippocampus and temporal lobe continue to lose volume. The authors showed that a relationship between the hippocampal loss in the presence of the Apolipoprotein E (ApoE) controlled for the Ikaros family of transcription factors and decreased volume of cerebrospinal fluid (CSF) supported the concept that increased hippocampal loss is an indicator of Alzheimer's disease pathology. Moreover, ApoE was a potential marker for early disease prevention therapies (ibid).

Schuff, et al. (2009) described the Alzheimer's Disease Neuroimaging Initiative (2009) that statement identification of patients who have MCI and decreased hippocampal size are four times more likely to progress to dementia over a truncated time period than patients whose hippocampus was of average size. The hippocampus in a healthy patient shrinks at about 1% per year, compared to 5% per year in a person with dementia. Thus, NeuroQuant® data evaluation provides an early warning before the onset of dementia. NeuroQuant® data allows monitoring of brain structure volumes over time to assess the progression of neurodegeneration.

Inflammatory Cytokines

Complement 4a (C4a) was one of 11 proteins identified by Bennett et al. (2012) as a potentially significant biomarker of neuronal injury. C4a is one of 30 proteins that comprise the complement system. The complement system circulates in the blood and becomes activated in response to infection in both serum and plasma by binding covalently to pathogens and

opsonizing them for engulfment by phagocytes bearing receptors for complement. The small fragments of complement proteins act as chemoattractants to recruit more phagocytes to complement activation. The terminal complement components damage bacteria by creating pores in the bacterial membrane, leading to non-specific defense against microbial infections and clearance of immune complexes and injured cells. In normal conditions, complement is tightly controlled by several fluid-phase and cell surface proteins to avoid injury to autologous tissues. When complement is activated, as occurs in autoimmune diseases or subjects with dysfunctional regulatory proteins, it drives a severe inflammatory response in numerous organs. Complement activation is regulated through activation of inhibitory proteins. When the regulatory mechanisms are inadequate, potential tissue damage results. C4a is increased in the brain and circulating blood, further increasing permeability of the BBB.

Khan et al. (2016) performed a systematic review of 11 research study papers (?) to identify specific proteins found in postmortem blood serum and brain of subjects with a previous Alzheimer's Disease and controls. Of the 371 proteins identified, only C4a was found multiple times in both the blood and brains of subjects with Alzheimer's Disease and controls. The significance of these findings is unclear because the authors stated that C4a may have potential as a serum biomarker for brain neuronal injury. Future research opportunities include correlating C4a with NeuroQuant® data analysis to assess neuropathologic changes.

Montagne, Zhao & Zlokovic (2017) reviewed human studies demonstrating BBB breakdown and neuronal dysfunction in dementia. The authors noted that increased CNS cerebral microbleeds reflected loss of cerebrovascular integrity as shown by MRI studies in 25% of individuals with MCI and 45–78% of individuals diagnosed with early Alzheimer's Disease. In addition, MMP-9 was identified as an essential mediator of BBB tight junctions and basement

membrane proteins resulting in BBB breakdown. Degradation of the BBB results in not only fibrinogen capillary leakage, but also in plasma proteins entering the neuroglial space and becoming neurotoxic.

Ramanet al., (2019), through the third-generation cohort of the Framingham Heart Study, identified serum VEGF as an indicator of small vessel disease and neurodegeneration in middle-aged adults. Elevated VEGF was also associated with lower total brain volume, as seen in a volumetric MRI scan.

The use of blood serum biomarkers and sensitive neurovascular imaging as diagnostic tools to identify pathogenesis of dementia before the transition to cognitive decline could promote early diagnosis, with possible adherence to lifestyle modifications to prevent or treat comorbid conditions, including hypertension and Type 2 diabetes mellitus. Ngandu et al. (2015) identified that the potential intervention window to delay the onset of dementia is during the MCI stage. Ngandu et al. (2015) estimated that 33% of dementia cases could be prevented if risk factors were addressed in the SCI stage.

Critique of the Literature

The Let Evidence Guide Every New Decision (LEGEND) is an evidence evaluation system that was used to grade the strength of the reported recommendations in the literature (Clark, Burkett, & Stanko-Lopp, 2009). This evaluation system was "created to provide tools for the primary care clinicians and assist them in synthesizing evidence and developing care recommendations based on published studies" (Clark, Burkett, & Stanko-Lopp, 2009, pg. 1058). The LEGEND evaluation criteria to determine strength of a body of evidence include quality (the aggregate of quality ratings), quality (magnitude of the effects of the numbers of studies), and consistency (extend of similar findings reported using a variety of study designs) (Clark, Burkett,

& Stanko-Lopp, 2009). The LEGEND system grades the strength of recommendations as follows: grade for the body of evidence; safety versus harm; health benefit to patient; the burden of the patient to adhere to recommendations: cost-effectiveness; the directness of the evidence; and impact on morbidity/mortality of quality of life (Clark, Burkett, & Stanko-Lopp, 2009).

Evaluation criteria for determining the strength of a body of evidence includes three well-established variables: quality (the aggregate of quality ratings for individual studies), quantity (magnitude of the effect of the numbers of studies), and consistency (the extent to which similar and different study designs) (Clark, Burkett, Stanko-Lopp, 2009. The levels of individual studies by domain, study design, and quality were graded as a = "good quality study" and b = "lesser quality study". Grades of high, moderate, low, or grade not assignable are used to describe the quality of evidence. The high grade indicates there are a sufficient number of high-quality studies with consistent results. Assigning a moderate grade means there are either multiple studies of lesser quality or with inconsistent results or a single well-done study. The low grade reflects a local opinion, case reports, case studies, and general reviews.

Using the LEGEND algorithm, this body of evidence scored "Moderate – High" due to the research designs with evidence ratings a follows: (1a – 1 b) for systematic review by Allison et al., (2019); Bennett et al., (2012); England et al., (2014); Khan et al., (2016); Ngandu et al., (2015); Noz et al., (2018); Ochs (2015); Schuff et al., (2009); Yu et al. (2014); and consensus statements (5a – 5b) by Kalaria, (2016); Leandrou (2018); Montagne et al. (2017); Ramen et al., (2019); and Sweeney (2019).

Rationale for Project

Multiple studies' results showed that the pathological changes of brain neuronal injury may begin many years before diagnosis of dementia (Braak & Braak, 1997; Crystal et al., 1988;

Villemagne et al., 2013, Teijido & Cacabelos, 2018, Van Bulck et al., 2019). Clinical importance of identifying serum biomarker(s) and MRI-detected brain changes for early diagnosis of neuronal injury stages is consistently discussed in the literature.

Chapter III

Methods

Research Question and Hypothesis

The research question is, "What are the symptoms of brain neuronal injury, symptoms, brain MRI with NeuroQuant® data, and molecular proteomic biomarkers associated with subjective cognitive impairment (SCI) and neuronal injury in adults?"

Two hypotheses will be tested in this study: 1) there will be a statistically significant positive increase in the levels of proteomic plasma biomarkers in the presence of SCI; 2) There will be a positive correlation between SCI and brain MRI with NeuroQuant® data.

Aims

Specific aims for this scholarly project are to:

a) Examine correlations among brain MRI with NeuroQuant® data and proteomic biomarkers (TGFβ1, MMP9, VEGF, and C4a) in adults with SCI.

b) Examine correlations among brain MRI with NeuroQuant® data and symptoms (fatigue, poor focus and concentration, poor memory, poor assimilation of new knowledge, word recollection difficulties, and confusion) in adults with SCI.

c) Examine correlations among sociodemographic data (age, gender, ethnicity, and race) and proteomic biomarkers (C4a, MMP-9, TGFβ1, and VEGF), and brain MRI with NeuroQuant® data in patients with SCI compared to age-matched controls.

Design

A retrospective study design using secondary data analysis was used to examine the interrelationships among NeuroQuant® data, symptoms, and proteomic biomarkers in patients with SCI. Aged matched controls were used for NeuroQuant® data, symptom and proteomic data.

Inclusion and Exclusion Criteria

Inclusion criteria were a) age 30 to 59 years old; b) patients established in the clinical practice between 2010 and 2015; c) patients with subjective cognitive impairment; d) symptom cluster checklist present in the patient's electronic medical record; e) NeuroQuant® data present in the electronic medical record; f) blood-borne biomarker data present in the electronic medical record.

Exclusion criteria was prior history of diagnosed traumatic brain injury (TBI).

Setting

The project setting was a primary care clinic located in a rural area in Southern Maryland. The clinical site team included one primary care physician and one office manager. The office manager supervises the reception desk, handles all billing, and has overall management of the clinic.

Sample Plan

A convenience sample was used composed of patients ranging in age from 30 to 59 years old, who were seen in the medical practice clinical site located in a Mid Atlantic urban area between 2015-2019. The principal investigator (PI) conducted in consultation with the scholarly project statistician the secondary data analysis of de-identified data abstracted from patients' medical records maintained at the project clinical site.

Instrument

All project data had previously been entered into the scholarly project data Excel spreadsheet by the office manager. The scholarly project research instruments included a) Sociodemographic Data; b) Proteomic Biomarker Data; c) NeuroQuant® data; and d) visual contrast test (VCS) symptom data.

Procedures

The PI presented the study rationale and planned procedures to the study clinical site leadership team and received written permission to conduct the project at the clinical site. The PI obtained written permission from the clinic site leadership for full access to both EMR and paper chart data. Upon IRB approval from the academic institution associated with this study, chart data will be accessed from the electronic medical record.

Data Collection Process

The clinical site office manager collected study specific retrospective data from the medical record by creating a query for patients seen between 2015 and 2019 before study implementation. The clinic site office manager extracted de-identified retrospective data from the medical record.

Data Management

The office manager entered all study data into one study Microsoft Excel database on the study-designated laptop computer at the clinic site. The database was uploaded into the project BOX by the PI, which is the password-protected online data-sharing platform maintained within the Georgetown University secure network system. Data was downloaded into the Statistical Package for Social Sciences (SPSS) computer software by the scholarly project statistician

maintained on the PI's password-protected personal computer. All data are maintained confidentially on this computer.

Data Cleaning

The PI and project primary mentor performed data cleaning before data analysis.

Statistical Analyses

Descriptive statistics, including frequencies, percentages, and central tendency measures, were used to provide a sociodemographic and clinical profile of the sample. Sample size justification for a two-tailed independent samples t-test comparing NeuroQuant® data to self-reported symptoms requires N = 352, alpha = 0.05 to yield a power = 0.80 with a small to medium effect size of d = 0.3. Sample size justification for a two-tailed Pearson's correlation analysis of NeuroQuant® data and proteomic biomarkers requires N = 193, alpha = 0.05 to yield a power = 0.80 with a small to medium effect size of r = 0.2. Minimum sample size required for linear regression with 15 predictors, alpha = .05, power = .80, is 389.

Human Subjects Considerations

The project design is a descriptive, correlational study using secondary data analysis with data maintained on the clinical site electronic medical records. There was no human subject interaction. The Georgetown University Institutional Review Board (IRB) gave approval for this study before any study activities were conducted. Deidentified participant data-maintained confidentiality on the PI's password-protected laptop computer. Identifiable data was protected under the Health Insurance Portability and Accountability Act (HIPAA) of 1996. Only the PI, faculty mentor, and the project statistician had access to the data. Study data were shared with the project team electronically through Georgetown University BOX for statistical analysis.

Confidentiality of all study data was maintained through the use of an individual participant identification number by the study site office manager. One list of the matching study chart number, electronic medical record number, and participant identification number-maintained confidentiality in the one medical co-investigator's locked office in a locked file. cabinet in the physician's locked office. To further ensure confidentiality, the data will be reported in aggregate form only.

Ethical Considerations

The PI obtained approval for the study from the Georgetown University (GU) Institutional Review Board (IRB) before any study activities were conducted.

Chapter IV

Results

This chapter presents the results of the data analysis, which is organized into three sections: a) the first section describes the relationship between proteomic biomarkers and volume percent intracranial volume (ICV) from the MRI with NeuroQuant® data; b) the second section compares brain volume percent by ICV by symptom; and c) the third section presents brain volume percent IVC by age and gender.

Sociodemographic Characteristics of the Sample

As an exploratory analysis of NeuroQuant® data has not been done previously. In this sample, a total of 250 NeuroQuant® data sets were extracted, 195 female and 55 males. Only 130 patients had all four proteomic biomarkers (C4a, MMP-9, TGFβ1, and VEGF), NeuroQuant® analysis, and symptoms. Therefore, it was appropriate to utilize all the data available for each comparison rather than restricting the sample to only those with all biomarkers. The sample size differs for each test and is noted in Table 1.

Table 1 Age Group Distribution

Age Group			
Frequency	Percent	Valid Percent	Cumulative Percent
30-34 years	19	7.6	7.6
35-39 years	29	11.6	11.6
40-44 years	54	21.6	21.6
45-49 years	84	33.6	33.6
50-54 years	27	10.8	10.8
55-59 years	37	14.8	14.8
Total	250	100.0	100.0

Aims

Aim 1 – Examine correlations among brain MRI with NeuroQuant® data and proteomic biomarkers (TGFβ-1, MMP9, VEGF, and C4a) in adults with SCI.

First, we examined the continuous variables (proteomic serum biomarkers and NeuroQuant® data) to test for normality. The normal distribution is an assumption for all parametric statistics, such as t-tests. Based on the Shapiro Wilks test of normality, none of the variables except for cerebellum volume LH & RH were normally distributed, therefore, nonparametric Mann Whitney tests were used to compare means rather than t-tests, and Spearman's correlation was used rather than Pearson's correlation.

The sample consisted of 130 patients with all four proteomic biomarkers. It was appropriate to utilize all the data available for each comparison rather than restricting the sample to only those with all biomarkers. Therefore, the sample size differs for each test and is noted in Table 2.

Table 2 Spearman's Correlations Between Proteomic Biomarkers and Volume Percent ICV from MRI

	C4a (n = 189) rho (p)	TGFβ1 (n = 216) rho (p)	MMP9 (n = 209) rho (p)	VEGF (n = 170) rho (p)
Forebrain LH	-.215 (.003)	-.015 (.832)	-.109 (.116)	-.049 (.528)
Forebrain RH	-.211 (.004)	.012 (.856)	-.113 (.104)	-.037 (.629)
Cortical Grey LH	-.067 (.356)	.028 (.677)	.008 (.906)	-.118 (.125)
Cortical Grey RH	-.068 (.350)	.038 (.576)	.036 (.605)	-.103 (.180)
Lateral Ventricle LH	-.135 (.063)	.056 (.413)	-.018 (.791)	.026 (.738)

Lateral Ventricle RH	-.235	-.032	.017	-.046
	(.001)	(.637)	(.807)	(.552)
Inferior Lateral	.227	-.030	.062	-.017
Ventricle LH	(.002)	(.666)	(.374)	(.828)
Inferior Lateral	.160	.064	.050	.087
Ventricle RH	(.028)	(.353)	(.468)	(.258)
Hippocampus LH	-.183	.101	.022	.008
	(.011)	(.141)	(.753)	(.917)
Hippocampus RH	-.067	.158	.070	.180
	(.362)	(.020)	(.315)	(.019)
Amygdala LH	-.033	.012	-.110	-.258
	(.652)	(.863)	(.113)	(.001)
Amygdala RH	.061	.013	-.111	-.101
	(.40)6	(.851)	(.109)	(.188)
Caudate LH	.046	.168	-.052	.050
	(.529)	(.014)	(.451)	(.515)
Caudate RH	-.022	.195	-.026	.030
	(.767)	(.004)	(.715)	(.699)
Putamen LH	.054	.006	-.007	-.111
	(.464)	(.934)	(.920)	(.149)
Putamen RH	.060	-.029	-.002	-.079
	(.409)	(.671)	(.973)	(.306)
Pallidum LH	.195	.093	.106	.064
	(.007)	(.172)	(.126)	(.427)
Pallidum RH	.163	.074	.027	.082
	(.025)	(.278)	(.695)	(.288)
Thalamus LH	.105	.088	.073	.120
	(.151)	(.199)	(.292)	(.120)
Thalamus RH	.164	.063	-.027	-.054
	(.025)	(.360)	(.703)	(.483)
Cerebellum LH	-.002	.055	.019	.005
	(.982)	(.419)	(.789)	(.951)
Cerebellum RH	.005	.054	-.015	.015
	(.950)	(.433)	(.829)	(.849)

Note: ICV= Intracranial volume, MRI= Magnetic resonance imaging, RH = right hemisphere, LH = left hemisphere

Complement 4a

There were eight areas of the brain significantly related to levels of Complement 4a (C4a): forebrain, lateral ventricles, inferior lateral ventricles, hippocampus, thalamus, and pallidum.

Upregulated serum C4a levels were significantly associated with decreased LH forebrain volume, rho = -.215, p = .003, decreased RH lateral ventricle volume, rho = -.235, p = .00l, decreased LH hippocampus volume, rho = -.183, p = .011.

Upregulated serum C4a levels were significantly associated with increased LH inferior lateral ventricle volume, rho = .225, p = .002, RH inferior lateral ventricle volume, rho = .160, p = .028, LH pallidum volume, rho = .195, p = .007, RH pallidum volume, rho = .163, p = .025, and increased RH thalamus volume, rho = .164, p = .025.

TGFβ-1

Increased serum TGFβ-1 levels were significantly associated with increased RH hippocampus volume, rho = .158, p = .020. LH caudate volume, rho = .168, p =.014, and RH caudate volume, rho = .195, p = .004.

MMP9

There were no significant correlations between MMP9 and any brain area volumes.

VEGF

Increased serum VEGF levels were significantly associated with increased RH hippocampus volume, rho = .180, p = .019 and a decreased LH amygdala volume, rho = -.258, p .001.

Aim 2 - Examine correlations between brain MRI with NeuroQuant® data and fatigue, poor focus and concentration, poor memory, poor assimilation of new knowledge, word recollection difficulties, and confusion in adults with SCI.

To examine this aim, we used the Mann Whitney test to compare mean NeuroQuant® volume percent for each brain area between subjects who exhibited a symptom and those who did not. The frequency distribution of symptoms is reported in Figure 1.

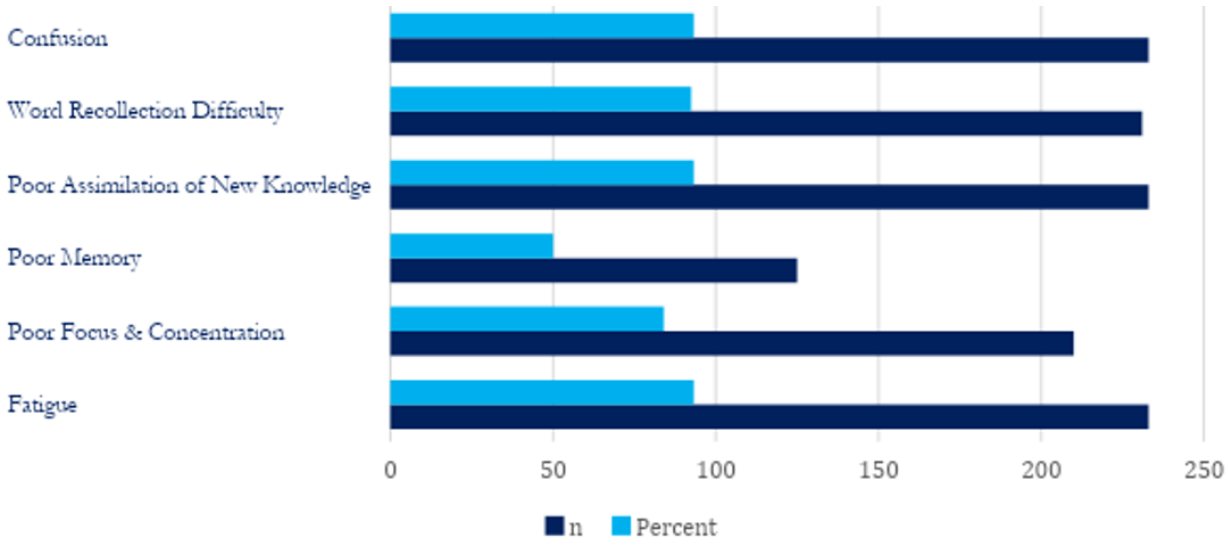

Figure 1 Distribution of Symptoms

The majority of subjects reported confusion, word recollection difficulty, poor assimilation of new knowledge, poor focus and concentration, and fatigue. Only 50% of the subjects reported poor memory as a symptom. This distribution of symptoms would lead us to expect no significant differences in volumes because almost all subjects reported these symptoms and there were not enough non-symptomatic subjects for comparison. Therefore, we only examined differences for poor memory. Our findings are noted in Table 3 and are significantly associated with a decreased volume in the LH cortical grey, LH inferior lateral ventricle, LH and RH hippocampus, and the LH and RH cerebellum.

Table 3 Comparison of Volume % by intracranial volume (IVC by symptom (n=250)

	Poor Memory Z (p)
Forebrain LH	-1.134(.217)
Forebrain RH	-1.357(.175)
Cortical Grey LH	-1.976(.048)
Cortical Grey RH	-1.458(.145)
Lateral Ventricle LH	-0.177(.860)
Lateral Ventricle RH	-0.817(.414)
Inferior Lateral Ventricle LH	-2.392(.017)
Inferior Lateral Ventricle RH	-0.866(.387)
Hippocampus LH	-2.469(.014)
Hippocampus RH	-2.176(.030)
Amygdala LH	-0.188(.851)
Amygdala RH	-0.815(.415)
Caudate LH	-1.206(.228)
Caudate RH	-0.221(.825)
Putamen LH	-0.202(.840)
Putamen RH	-0.267(.789)
Pallidum LH	-0.603(.546)
Pallidum RH	-1.115(.265)
Thalamus LH	-0.751(.453)
Thalamus RH	-0.835(.404)
Cerebellum LH	-2.419(.016)
Cerebellum RH	-2.303(.021)

Note: based on Mann Whitney (Z reported because Z associated with Mann Whitney is easier to interpret than U – remember that Z is the standard normal distribution with z = ±1.96 having a p = .05). ICV = intracranial volume, RH = right hemisphere. LH = left hemisphere.

Aim 3 - Examine relationships between demographics and serum proteomic biomarkers and NeuroQuant® data in patients with SCI compared to age-matched controls.

There were only 18 aged-matched controls, and thus, this aim could not be met. However, we did examine the relationships between the demographic variables of age and gender and NeuroQuant® data. There were too many missing race and ethnicity variables for analysis. For age, Spearman's Correlations were used. For gender, Mann Whitney was used. Table 4 shows mean volumes by gender for the areas with significant differences

Age: There were significant decreases in volume with age in the following areas: LH forebrain, LH amygdala, LH & RH putamen, LH & RH pallidum, and RH thalamus. There were significant increases with age in the following areas: LH & RH lateral ventricle and RH hippocampus.

Gender: There were significant differences by gender for the following areas: LH and RH forebrain, RH hippocampus, LH caudate, and LH thalamus Table 4 shows mean volumes by gender for the areas with significant differences. Males had higher volumes than females for forebrain LH and RH, caudate LH, and thalamus LH. Females had higher volumes than males for hippocampus RH.

Table 4 Brain Area Volume % ICV by Age and Gender

	Age (n = 250)		Gender (n = 250)	
	rho	P	Z	p
Forebrain LH	-.118	.061	-3.427	.001
Forebrain RH	-.050	.427	-2.529	.011
Cortical Grey LH	-.054	.394	-0.829	.407
Cortical Grey RH	-.007	.910	-0.850	.395
Lateral Ventricle LH	.308	< .001	-1.833	.067
Lateral Ventricle RH	.280	< .001	-1.826	.068
Inferior Lateral Ventricle LH	-.004	.954	-0.138	.891
Inferior Lateral Ventricle RH	.059	.352	-1.599	.110
Hippocampus LH	.074	.242	-0.009	.993
Hippocampus RH	.163	.010	-3.151	.002
Amygdala LH	-.135	.033	-1.304	.192
Amygdala RH	.008	.896	-0.588	.557
Caudate LH	.042	.511	-2.309	.021
Caudate RH	.012	.850	-1.624	.104
Putamen LH	-.223	< .001	-0.152	.880
Putamen RH	-.285	< .001	-1.550	.121
Pallidum LH	-.182	.004	-0.219	.827
Pallidum RH	-.133	.035	-0.069	.945
Thalamus LH	-.037	.556	-2.590	.010
Thalamus RH	-.134	.034	-0.781	.435
Cerebellum LH	-.015	.818	-1.045	.296
Cerebellum RH	-.007	.916	-1.498	.134

Note: significance levels for age based on Spearman's correlation; significance levels for gender based on Mann Whitney. ICV = Intracranial volume. LH = left hemisphere. RH = right hemisphere.

Chapter V

Discussion

The research question was "What are the symptoms, brain MRI with NeuroQuant® data, and serum proteomic biomarkers associated with SCI and neuronal injury in adults with subjective cognitive impairment?" The study's primary aim was to examine correlations among brain MRI with NeuroQuant® data and proteomic biomarkers (C4a, MMP9, TGFβ1, and VEGF) in adults with SCI.

Complement C4a, an inflammatory marker in both the blood and brain, was significantly correlated with architectural changes throughout the brain parenchyma. Notably, upregulation of serum C4a was associated with atrophic changes in the forebrain, the lateral ventricle, and the hippocampus. Our findings support the hypothesis that complement C4a negatively impacts the integrity of the BBB. Dysfunction of this critical barrier occurs in brain neuronal injury and possibly in subsequent neurodegenerative diseases. Our data suggests upregulated C4a contributes to BBB disruption and may be implicated in subjective cognitive impairment.

Transforming Growth Factor β1 (TGFβ1) has pleiotropic functions in various organs, regulating growth, differentiation, and survival of different cell types. Considered both a growth factor and neuroprotective, TGFβ1 influences the immune system and is also considered a cytokine. Our findings are congruent with the literature suggesting TGFβ1 excess was seen in brain neuronal injury and neurodegenerative diseases and implicated in their pathogenesis (Grammas & Ovase, 2002). With this background, we speculate that the upregulation of TGFβ1 in brain neuronal injury may represent an adaptive response. Glucose intolerance increases the brain's availability of glucose and assists in molecular stress management (Peters, McEwon, &

Friston, 2017). However, when induction of glucose intolerance is chronic, it lowers the threshold of developing mitochondrial disorders. As discussed by Shoemaker (2020), molecular hypometabolism depends on the dual role of energy conservation versus energy expenditure to support cell proliferation. The observation of the cortical grey, hippocampal, and cerebellar atrophy on the NeuroQuant® is expected in MCI and patients diagnosed with dementia. Future research should examine the potential utility of TGF-β1, molecular hypometabolism, and the downstream mechanism as a viable target for treatment.

Vascular endothelial growth factor (VEGF) is essential for maintaining the vasculature's optimal function. Our finding verifies VEGF as a candidate for detecting vascular alterations in acquired brain neuronal injury. Essential for maintaining the cerebral vasculature optimal function, upregulated VEGF may induce pathological vessel formation through angiogenesis. Locally produced and secreted by astrocytes, VEGF regulates neuronal cell survival, angiogenesis, and vascular cell permeability (Koch & Claesson-Welsh, 2012; Rosenstein, Krum, & Ruhrberg, 2010). Additionally, VEGF's downregulation was reported in cerebral capillaries in postmortem brain tissue derived from patients with neurodegenerative diseases, indicating pathological vessel formation (Provias & Jernes, 2014).

The second aim was to examine correlations between brain MRI with NeuroQuant® data and symptoms in adults with SCI. The majority of patients suffered from fatigue, poor focus and concentration, poor assimilation of new knowledge, word recollection difficulty, and confusion. However, only half of the subjects reported poor memory as a symptom which was associated with atrophy of the hippocampus and cerebellum, two key indicators of Alzheimer's disease.

We observed an interesting interaction between C4a, and the baseline hippocampal volume driven by a strong association in SCI participants reporting poor memory, fatigue, poor

focus and concentration, poor assimilation of new knowledge, word recollection difficulties, and confusion. Although the study was somewhat underpowered to fully investigate biomarkers, NeuroQuant® data, and SCI symptoms, the identified diagnostic interaction and the stratified results suggest that C4a, TGFβ1, and VEGF have relevance in individuals at highest risk for future neurodegeneration.

The tertiary aim was to examine relationships between the sociodemographic data (age, gender, ethnicity, and race) and proteomic biomarkers (C4a, MMP-9, TGFβ1, and VEGF), and brain MRI with NeuroQuant® data in patients with SCI compared to age-matched controls. Because there were only 18 aged matched controls, this aim could not be met. However, we did examine the relationships between the demographic variables of age and gender and NeuroQuant® volumes.

Cellular and molecular changes naturally occur during normal aging and may render specific neurons vulnerable to degeneration. During the normal aging process, brain cells undergo changes in oxido-reduction (redox) reactions and experience increasing levels of oxidative stress, perturbed energy homeostasis, and accumulation of damaged proteins and lipid membranes (Mattson & Magnus. 2006). Aging is associated with decreases in mitochondrial function, increased vulnerability of mitochondria to biotoxins or endotoxins, and impaired neuronal glucose uptake into the cell which compromises the brain's ability to maintain ion homeostasis and other energy-dependent cellular processes (Keller et al., 1997). Many of the age-related deficits in energy metabolism might be a consequence of oxidative stress (Ames, Shigenaga, and Hagen, 1993). Excessive oxidative stress may in turn provide an unfavorable cellular environment that puts individuals at increased risk. Aging is a primary risk factor for

neurodegeneration, and oxidative stress has been implicated in the pathogenesis and progression of neurodegeneration disease like Alzheimer's (Ames, Shigenaga, Hagen, 1993; Beal, (1995).

Our project identified multiple areas of atrophic changes in adults aged 30 to 59 years old. Notably, the forebrain, amygdala, putamen, and the thalamus are significantly smaller with age. The effects of aging on the brain has multiple potential etiologies. Aging has its effects on the molecules, cells, vasculature, and gross morphology. Genetics, neurotransmitters, cell hypometabolism, mitochondrial dysfunction, hormones, and environmental interactions all have a part to play in brain aging. Higher levels of education or occupational attainment may act as a protective factor. Also protective are a healthy diet, low to moderate alcohol intake, and regular exercise. The aging brain also suffers from cell hypometabolism and reduced glucose and oxygen input as vascular efficiency fails. This is an area for future scholarship.

Gender differences in volume loss in the brain were widespread in our data set. It is known that sex hormones can affect cognitive processes in adulthood and that changes in sex hormones occur in aging, particularly in women during the perimenopause and menopausal transition in the female brain. Future research is needed to elucidate the facilities to and barriers to positive aging. Having a comprehensive understanding of the cellular metabolic and respiratory profiles among chromosomal sex traits can help identify potential therapeutic targets and windows of opportunities for testing and treatment of brain neuronal injury.

Limitations

There are numerous limitations of this scholarly project. The study design was cross sectional, and thus, results are limited to only one time point. The retrospective data collection was conducted at one primary care clinic, limiting its generalizability to only the study clinic. Also, the lack of age-matched control data significantly impacted the results.

Strengths

This retrospective study had multiple strengths built into the design to include access to an organized data source. The unexpected SARS-CoV-2 pandemic disrupted research globally. Using a retrospective, secondary data source allowed for the generation of new knowledge during an extended period of social distancing.

Generalizability of Results

The small sample size and use of one clinical site limits the study results to only this clinical study site.

Implications for Scholarship, Education, and Practice

Research emphasis has recently been focused on discovering unique proteomic markers in the peripheral blood circulation that are indications of central brain changes. These data suggest that pathologies associated with neuronal injury present in the brain are also in the plasma. The proteomic biomarkers C4a, TGFβ1, and VEGF are potential peripheral indicators of neuronal damage. Early screening for acquired neuronal injury is clinically essential to allow prompt interventions and prevent mild cognitive impairment progression to mild cognitive impairment. Adopting the easy-to-order NeuroQuant® and widely available proteomic biomarkers is the first step in adopting a screening process.

Future scholarship should include examination of estrogen as a master regulator of brain glucose metabolism, mitochondrial function and cellular hypometabolism. Genetic factors such as mitochondrial genetic variance, APOE genotype, and chromosomal sex difference should also be explored.

Routine screening for brain neuronal injury is appropriate in the primary care settings. Clinical research is needed to evaluate the benefits of early screening of 30- to 59-year-old adults

with poor memory as a vascular phenomenon. Research and education for health care providers are required to distinguish the difference between memory impairment and psychological disorders, which frequently occur together and share similar phenomenological features. The positive benefits of identifying early brain neuronal injury diagnosis to the patient include clarifying and validation of the symptoms. Early diagnosis and subsequent access to lifestyle changes and nonpharmacological interventions may delay or prevent progression of subjective cognitive impairment to cognitive decline as demonstrated in the 2-year observational FINGER study (2015). Ngandu et al. (2015) demonstrated a 25 to 150% improvement in cognition due to non-pharmaceutical interventions, a 25% improvement in overall cognition, 83% improvement in executive function, and a 150% improvement in processing speed with diet and lifestyle alterations. Further, vascular disease may share risk factors, including hypertension, type 2 diabetes mellitus, smoking, diet, and exercise habits. This has led the WHO (2019) to recommend the combined implementation of their recent guidelines on risk reduction of cognitive decline with interventions related to managing risk factors for cardiovascular disease and diabetes.

There also are numerous potentially reversible causes of poor memory. Patients with an abnormal NeuroQuant® data may have treatable conditions that could slow down further brain damage and possibly the development of dementia when controlled. These include hypertension, diabetes, hyperlipidemia, smoking, and atrial fibrillation (Pantoni, 2010). Both heart failure and chronic obstructive pulmonary disease are associated with an increased risk of cognitive impairment (Singh, Mielke, Parsaik, Cha, Roberts, & Scanlon, 2014). This suggests early treatment and improvement of oxygenation may slow the development of cognitive impairment. Persons with diabetes mellitus have an early onset of cognitive impairment and a higher

likelihood of developing dementia (Morley et al., 2016). Hyperglycemia has been associated with reversible cognitive impairment in humans and animals (Reaven, Thompson, Nahum, & Edmund, 1990).

The significant economic burden of advanced acquired brain neuronal injury is reported primarily in three main sectors: healthcare; social care; and informal care. The majority of costs fall on informal caregivers. Everyday care costs relate to family providing unpaid care for people living with advanced neuronal injury. Recognition and management of risk for the all-cause neuronal injury earlier in adult life are essential to have the most significant impact. Individuals with these risk factors can be advised to improve lifestyle choices and control their modifiable risk factors to minimize their risk for future dementia and other chronic neurodegenerative conditions. Also, brain health should be protected throughout life by avoiding alcohol and substance abuse, supporting lifelong learning, social interaction, and stimulation later.

Primary care workers and other healthcare professionals in contact with people with mild cognitive impairment should routinely ask questions as part of their standard patient review to identify symptoms. Primary care providers are ideally positioned to monitor patients at risk of dementia as they have access to an individual's medical and family history. However, recognition is not just the responsibility of general practitioners but also other professionals who have regular contact with patients and may notice changes in cognitive functioning, including community pharmacists, nurses, and social care.

Conclusions

This study examined the impact of proteomic biomarkers, NeuroQuant® data, and brain neuronal injury symptoms in one clinic setting. The central hypothesis was that there would be a

positive increase in proteomic plasma biomarkers' levels with volumetric changes observed in the brain MRI with NeuroQuant® data in adults with SCI. Atrophy to the forebrain, lateral ventricles, hippocampus, and amygdala was observed in the presence of upregulated C4a, TGFβ1, and VEGF. There was a positive correlation between SCI symptoms and volumetric atrophy to the brain, specifically the hippocampus and cerebellum.

A key feature seen with cerebral neuronal injury is chronic inflammation, whether occurring primarily as part of the disease or as the body's response to the pathological effects. Our study results validate brain neuronal injury as an inflammatory process. While the mechanism underlying brain neuronal injury remains undetermined, it is clear during the 20-year prodromal period, mitochondrial dysfunction and brain molecular hypometabolism are evident.

Future scholarship should elucidate the chromosomal sex differences in brain neuronal injury, the impact of the female brain's perimenopausal transition to develop a comprehensive understanding of the dynamic metabolic aging process, potential interventions, and possible window of prevention for brain neuronal injury. The availability of aged-match controls will help future scholarly work examine the relationship between NeuroQuant®, age, and gender. Conceivably, serum proteomic biomarkers C4a, TGFβ1, and VEGF and the NeuroQuant® could act as a prescreening tool to signal neuropathological presence changes in the presence of subjective cognitive impairment in adults.

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