EXAMINING THE ASSOCIATIONS BETWEEN POST-STROKE COGNITIVE FUNCTION AND RECURRENT STROKE RISK FACTORS THAT INCLUDE CO-MORBID CONDITIONS

by

Melissa Michaels McElroy

Copyright © Melissa Michaels McElroy 2020

A Dissertation Submitted to the Faculty of the

COLLEGE OF NURSING

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

2020
THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Melissa Michaels McElroy, titled Examining the Associations between Post-Stroke Cognitive Function and Recurrent Stroke Risk Factors that Include Co-Morbid Conditions and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

_________________________ Date: __________________
Helena W. Morrison, PhD, RN

_________________________ Date: __________________
Ruth E. Taylor-Piliae, PhD, RN, FAHA, FAAN

_________________________ Date: __________________
Janet L. Rother, PhD, MS, MA

Final approval and acceptance of this dissertation is contingent upon the candidate’s submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

_________________________ Date: __________________
Helena W. Morrison, PhD, RN
Dissertation Committee Chair
College of Nursing
ACKNOWLEDGMENTS

For all those that have supported me in this endeavor, I want to offer my sincerest appreciation and deepest gratitude; it is only through God’s continued grace in my life that I have been able to achieve such accomplishments. First, to my children—Max and Mackenzie—everything I have done and continue to do, I do for them. I love you both so very much! Second, to the faculty that has contributed to my education but most of all, Dr. Helena Morrison, who has shared her passion for research and mentored me in a way that was profoundly personal and with the upmost compassion. And, to my committee, Dr. Janet Rothers and particularly Dr. Ruth Taylor-Piliae, for allowing me this opportunity to expand on her existing research. Third, to the remainder of my family and close friends who have been my support; you have been my constant. I had no idea how this process would eventually unfold and when I didn’t believe I would finish, each one of you reminded me what mattered most—the choice to keep pushing forward. Lastly, for all the patients that inspire this work, thank you for allowing student researchers such as myself the opportunity to learn from your hardship and contribute to science on your behalf.
DEDICATION

For Max and Mackenzie
# TABLE OF CONTENTS

LIST OF FIGURES ........................................................................................................... 8
LIST OF TABLES ............................................................................................................... 9
ABSTRACT ......................................................................................................................... 10

## CHAPTER 1: INTRODUCTION ......................................................................................... 12
Ischemic Stroke ............................................................................................................. 12
Significance of the Problem ......................................................................................... 14
Theoretical Model ......................................................................................................... 15
Susceptibility for Post-Stroke Cognitive Decline ......................................................... 17
  Demographic Variables: Risk Factors and Comorbidities ........................................ 17
  Non-modifiable risk factors. ....................................................................................... 17
  Comorbidities. ........................................................................................................... 18
Assessing Comorbidity ............................................................................................... 18
Mechanisms of Post-Stroke Cognitive Decline ............................................................ 19
Assessing Cognitive Function .................................................................................... 20
Study Purpose and Aims ............................................................................................... 21
  Aim 1 ......................................................................................................................... 21
    Aim 1a. ................................................................................................................... 21
    Aim 1b. ................................................................................................................... 21
  Aim 2 ......................................................................................................................... 21
  Aim 3 ......................................................................................................................... 21

## CHAPTER 2: LITERATURE REVIEW .............................................................................. 22
Inflammation .................................................................................................................. 22
  Cell Responders ....................................................................................................... 22
  Cytokines and Chemokines ....................................................................................... 24
  Small Molecular Messengers .................................................................................... 25
    Reactive oxygen species. ....................................................................................... 25
  Cell Signaling in Inflammation ............................................................................... 27
Ischemic Stroke ............................................................................................................. 28
  Primary Injury ......................................................................................................... 29
  Secondary Injury ..................................................................................................... 29
  Blood Brain Barrier Dysfunction .......................................................................... 31
  Immune cell responders. ......................................................................................... 32
Measuring Brain Injury after Ischemic Stroke .............................................................. 34
Inflammation: The Common Denominator of Ischemic Stroke, Cardiovascular Related
Diseases and Post-Stroke Cognitive Decline ............................................................... 36
Cardiovascular-Related Comorbidities ...................................................................... 37
  Hypertension. .......................................................................................................... 37
  Congestive heart failure. ......................................................................................... 38
TABLE OF CONTENTS – Continued

Diabetes mellitus. .................................................................39
Coronary artery disease and dyslipidemia. .................................39
Arrhythmias. ........................................................................41
Major depression. ................................................................41
Post Stroke Cognitive Decline .................................................42
Neurocognitive Assessment Tools ..............................................44
Montreal Cognitive Assessment ..............................................45
Mini-Mental State Examination ................................................46
Gender Differences ..................................................................47

CHAPTER 3: METHODS ................................................................50
Study Sample ...........................................................................50
Participant Recruitment .............................................................50
Inclusion criteria.......................................................................50
Exclusion criteria......................................................................51
Study Procedures ......................................................................51
Study Measures ........................................................................51
Recurrent Stroke Risk Measure ..................................................51
Cognitive Assessment Measures .................................................52
Data Analysis Plan ....................................................................53

CHAPTER 4: RESULTS ................................................................55
Characteristics of Participants and Data Set .................................55
Aim 1 Analysis ..........................................................................56
Aim 1: Evaluate the Relationship between Cognitive Function and Stroke Risk Factors ..................................................56
Aim 1a: Examine the associations between participants’ stroke comorbidities (overall SPI-II score) and overall cognitive function (MoCA and MMSE scores) ..................................................56
Aim 1b: Examine the relationships between overall stroke comorbidities and the cognitive individual domains captured by the MoCA and MMSE assessments. ..................................................56
Aim 2 Analysis ..........................................................................58
Aim 2: Examine Differences between Overall Cognitive Function and Individual Co-Morbid Conditions Represented in the SPI-II Assessment Tool .................................................58
Aim 3 Analysis ..........................................................................60
Aim 3: Determine if Post-Stroke Cognitive Function is Different According to Gender in This Population of Community-Dwelling Stroke Survivors .................................60
TABLE OF CONTENTS – *Continued*

CHAPTER 5: DISCUSSION .............................................................................................................61
Study Limitations and Strengths .................................................................................................67
Conclusions ................................................................................................................................68

APPENDIX A: THE UNIVERSITY OF ARIZONA INSTITUTIONAL REVIEW BOARD
APPROVAL LETTER ..................................................................................................................70
APPENDIX B: APPROVAL FOR USE OF SECONDARY DATA .................................................72
APPENDIX C: FUNCTIONAL STROKE SCALES .....................................................................74
APPENDIX D: COGNITIVE ASSESSMENT TOOLS .................................................................85
APPENDIX E: RECURRENT STROKE RISK PROFILE ..............................................................89
APPENDIX F: SUPPLEMENTARY DATA FIGURES .................................................................91
APPENDIX G: SUPPLEMENTARY PARAMETRIC ANALYSES ...............................................95

REFERENCES ............................................................................................................................98
LIST OF FIGURES

Figure 1  Summary of the Health Belief Model .................................................................16
Figure 2  The Influence of Pre-stroke Comorbidity Burden on Post-Stroke Cognitive Decline44
LIST OF TABLES

Table 1  Demographics and Comorbidities of Study Participants (N = 97) ..................55
Table 2  Nonparametric Correlations between MoCA Cognitive Function Domains and SPI-II.................................................................57
Table 3  Nonparametric Correlations between MMSE Cognitive Function Domains and SPI-II....................................................................................57
Table 4  Descriptive Table of MoCA and MMSE ............................................................................................................................59
Table 5  Median MoCA Scores and Stroke Comorbidities: Nonparametric Mann-Whitney U Test..................................................................................59
Table 6  Median MMSE Scores and Stroke Comorbidities: Nonparametric Mann-Whitney U Test..................................................................................60
Table 7  Average MoCA and MMSE Scores by Gender .........................................................................................................................60
ABSTRACT

Cognitive decline is a common yet elusive outcome following ischemic stroke. The immune system is believed to play a role in brain function and cognition, yet exactly how remains unclear. Following an ischemic brain injury, brain tissue is subject to both the deleterious effects of local inflammation and compromised structural integrity of the blood brain barrier, allowing circulating immune cells and inflammatory proteins to enter the brain and perpetuate neuroinflammation, tissue death and eventually neurodegeneration. Chronic inflammation in the brain and an injured blood brain barrier have been proposed as mechanisms of post-stroke cognitive decline. There is now an accumulation of evidence that suggest a number of cardiovascular-related comorbidities have also immune responses that contribute to systemic inflammation and thus, neuroinflammation mentioned above. The purpose of this secondary data analysis, collected from community-dwelling stroke survivors, was to evaluate the relationship between post-stroke cognitive decline and cardiovascular-related comorbid conditions, operationalized using a recurrent stroke risk score, the Stroke Prognosis Instrument-II (SPI-II). We then examined the relationship between SPI-II scores and measures of cognitive decline such as the Montreal Cognitive Assessment and the Mini-Mental State Exam (MoCA & MMSE, respectively) as well as differences in MoCA and MMSE scores according to stroke comorbidities. Our primary finding is that as total SPI-II score increased (our measure of comorbidity burden), MoCA and MMSE scores decreased (measures of post stroke cognitive decline; \( r = -0.25, p = 0.01 \) and \( r = -0.22, p = 0.03 \), respectively). Participants with medium SPI-II scores had lower MoCA scores than those with low SPI-II scores (Dunn’s post-hoc test, \( p = 0.05 \), adjusted; Cohen’s \( d = 0.43 \)). On the other hand, MoCA and MMSE scores were similar
when patients were stratified by singular comorbid conditions (e.g., those with & without hypertension). Interestingly, total SPI-II score correlated with a specific MoCA domain—delayed recall ($p=0.001$). Lastly, in this data set, post-stroke cognitive decline was not influenced by gender. Although this secondary data analysis has inherent limitations, these data support the premise for studying comorbidity burden and its effect on post-stroke cognitive decline.
CHAPTER 1: INTRODUCTION

Stroke is a leading cause of death and disability in the United States (U.S.) and worldwide (Mozaffarian et al., 2016). Among all cardiovascular diseases, stroke is the leading cause of physical disability, and is the second leading cause of mortality globally (Kleindorfer et al., 2010; Krishnamurthi et al., 2013). Based on U.S. Census Bureau estimates, there will be a proportional increase in aging adults through 2030, dramatically increasing stroke incidence as age is the leading risk factor for stroke (Ovbiagele et al., 2013).

Ischemic Stroke

Stroke is categorized into two clinical subtypes, ischemic and hemorrhagic. Hemorrhagic stroke accounts for about 10% of all strokes nationwide. Worldwide, of the more than 6 million stroke deaths, nearly half were hemorrhagic—an indicator that hemorrhagic stroke is deadly when it occurs (Lisabeth et al., 2015). However, ischemic stroke incidence far outweighs that of hemorrhagic strokes in the U.S. and while not nearly as deadly as hemorrhagic stroke, those that survive are left with debilitating functional and mental deficits. One such deficit is cognitive decline, a subject matter central to this research inquiry. Ischemic strokes account for 87% of stroke cases and are estimated to affect over 7 million Americans (Virani et al., 2020). In 2013 alone, there were over 25 million stroke survivors globally requiring post-stroke rehabilitation for stroke injury and post-stroke related complications (Benjamin et al., 2019; Benjamin et al., 2018; Feigin et al., 2015). About three-quarters of all strokes occur after the age of 65 (Yousufuddin & Young, 2019). Because age is the leading non-modifiable risk for stroke, as the nation’s population continues to age, the burden of stroke will also increase (Ovbiagele et al., 2013).
Ischemic stroke is defined as an acute vessel occlusion, resulting in reduced blood flow to any area of the brain and thereby dramatically reducing oxygen and nutrient delivery to brain cells. Without blood flow, ischemia ensues resulting in primary injury (i.e., excitotoxicity & calcium overload) and cell death (Dirnagl, Iadecola, & Moskowitz, 1999). Triggered by cell death, both local (within the brain parenchyma) and systemic (from the vasculature & blood) inflammatory responses expand brain injury rather than contributing to resolution (Dirnagl, 2012; Kataoka, Kim, & Plesnila, 2004; Nedergaard & Dirnagl, 2005).

Although many recover from functional and cognitive deficits after stroke, about one third of stroke survivors develop a delayed and progressive dementia that results in a cognitive decline, also known as vascular dementia (Pendlebury & Rothwell, 2009; Prencipe et al., 1997). In fact, for about 26% of stroke survivors, stroke leads to a 200 percent increase in a person’s risk of developing dementia that is related to a number of factors, including co-comorbid conditions that are common with stroke (e.g., hypertension, dyslipidemia & diabetes) (Ivan et al., 2004; Pendlebury & Rothwell, 2009). Interestingly, inflammation—a part of the body’s immune response to disease and injury—is an aspect of many stroke comorbidities. The health conditions that are often co-morbid to ischemic stroke are chronic, persist during stroke, contributing to post-stroke complications such as vascular dementia, and may be a contributing factor to post-stroke cognitive decline. Gender also plays a role in ischemic stroke recovery and post-stroke deficits. Women, compared to men have a worse post-stroke quality of life, particularly in the areas of cognition and mobility (Bushnell et al., 2014; Phan et al., 2017).

Preventing these secondary post-stroke complications in men and women starts with deciphering what puts a person at risk for cognitive impairment, a debilitating secondary
outcome. Unfortunately, the pathophysiology of post-stroke cognitive decline and what characteristically predisposes versus protects an individual from its effects are poorly understood. Few studies investigate the relationship between the presence of stroke co-morbid conditions and the occurrence of post-stroke cognitive decline from a framework of susceptibility. Individuals may be more susceptible to post-stroke cognitive decline when co-occurring, unresolved brain inflammation (post-injury) overlaps with the presence of comorbidities that are commonly observed in stroke patients.

**Significance of the Problem**

Ischemic stroke is a devastating disease with both acute and lasting outcomes. Every year, approximately 800,000 strokes occur in the United States, costing the country more than 33 billion dollars in both direct and indirect costs (health care services, medicines & days missed from work that occur in relation to stroke) (Benjamin et al., 2017). These constraints then translate to increased financial and logistical caregiving burdens to support stroke survivors (Skolarus, Freedman, Feng, Wing, & Burke, 2016). Ischemic stroke has only a singular treatment—clot removal—accompanied by a narrow therapeutic window that leaves many patients untreated. Equally limiting, the cognitive decline that may accompany stroke is poorly understood and without treatment. In a logistically strained healthcare system, the burden of stroke stresses the existing infrastructure of available neurologists, rehabilitation hospitals and caretakers, which then may increase nursing home demand (Howard & Goff, 2012). Therefore, stroke is a top national healthcare concern and nursing is one of the largest professions in the U.S. healthcare workforce, intimately responsible for the care of stroke patients both in and out of the hospital (United States Department of Labor, 2018).
Nursing at the forefront of healthcare will be responsible for managing the range of complications and long-term disability associated with stroke, to include cognitive decline. As such, the ultimate goal of this research is to determine the association between post-stroke cognitive declines in relation to existing chronic, co-morbid conditions, thereby highlighting those at risk for ischemic stroke complications. Moreover, an awareness of these associations can then assist in forming individual perceptions regarding risk and benefits, which in many cases provide the motivation needed to engage in preventative health behaviors (Rosenstock, 1974). Aside from using data as a motivational basis, independently using a theoretical framework can frame patient education and has proven to be an effective method in improving health outcomes (Venmans, Gorter, Hak, & Rutten, 2008).

Theoretical Model

In the 1950’s, public health researchers who were heavily influenced by the theories proposed by Kurt Lewin, worked collaboratively to develop a theory, the Health Belief Model (HBM), that would explain why otherwise asymptomatic people were unlikely to participate in preventative health behaviors (Rosenstock, 1974). Guided by this deductive reasoning, researchers were determined to not only address the apparent public health concerns (at that time, tuberculosis exposure) but also, theoretically frame why the vast majority failed to accept any preventative screening tests despite being offered at no cost. Its premise is based upon understanding what directs an individual to change their behavior, particularly motivation and perception (Rosenstock, 1974). The central tenets of the HBM include “perceived susceptibility, perceived seriousness, perceived benefits, perceived barriers, cues to action and self-efficacy” (Li, Stotts, & Froelicher, 2007).
In this current study, we use the HBM to guide our exploration of susceptibility of cognitive decline in the setting of ischemic stroke (Becker et al., 1978). The major concepts and their definitions as outlined by Rosenstock (1974, p. 330-331) include 1) “perceived susceptibility” or personal vulnerability towards the development of disease, 2) “perceived severity” or feelings toward the seriousness of having the disease, 3) “perceived benefits” or what the person believes about both availability and effectiveness of a particular course of action, and 4) “barriers to taking action” or any negative component impeding preventive health behaviors (Janz & Becker, 1984). Two additional concepts were later added to the model, “cues to action,” which describe a person’s thoughts or preference in triggering the decision-making process and “self-efficacy” or a patient’s confidence using a prescribed treatment despite the presence of barriers (Janz & Becker, 1984; Olsen, Smith, & Oei, 2008; Rosenstock, 1974). The relationships among these related concepts are shown in Figure 1.

**Figure 1**

*Summary of the Health Belief Model*

[Diagram showing relationships among concepts]

Modifying factors such as demographic and psychological variables (e.g. age, hypertension, dyslipidemia, and depression) are seen to have a direct influence on the patient’s perceived threat of disease, perceived seriousness of the disease, and any net benefits perceived
after barriers have been considered (Rosenstock, 1974). Although the modifying factors do not directly influence health behaviors, individual perceptions such as risk, severity, benefits and barriers predict motivation to engage in an action to prevent disease (Olsen et al., 2008).

Applying the HBM to this study, if stroke patients understood the modifying factors, specifically the co-morbid conditions, that make them susceptible to post-stroke cognitive decline, then they may be more likely to take action to prevent cognitive decline. In addition, current research suggest that inflammatory factors and immune responses increase the susceptibility for post-stroke cognitive decline. Therefore, our overarching hypothesis is the presence of co-morbid conditions, and the associated inflammatory milieu, will increase susceptibility to post-stroke cognitive decline. We aim to explore this association in the current study. Once elucidated, this knowledge may support future work to engage patients using the HBM toward preventative (or protective) behaviors to lessen inflammation, reduce susceptibility, and promote actions that will prevent or delay post-stroke cognitive decline (Becker et al., 1978; Wallace, 2002).

**Susceptibility for Post-Stroke Cognitive Decline**

**Demographic Variables: Risk Factors and Comorbidities**

**Non-modifiable risk factors.** Age is a leading risk factor for ischemic stroke. Stroke largely affects the older population (>65 years old), a demographic that will account for more than 20% of the total population by 2050 (Halaweish & Alam, 2015). Ischemic stroke risk doubles with each decade after age 55, with the average age of stroke onset being 71 in males and 75 in females, respectively (Hegen, Auer, & Deisenhammer, 2017).
Stroke outcomes are greatly influenced by gender. First seen in the Framingham epidemiology study, women have a disproportionately higher lifetime risk of stroke compared to men, originally attributed to longer life expectancies (Madsen et al., 2017). Stroke incidence appears to be greater for women starting after 71 years old compared to 68 years old in men (Appelros, Stegmayr, & Terent, 2009; Madsen et al., 2017). Women of advancing age are also reported to have poorer post-stroke functional outcomes. Epidemiological studies suggest the sexual dichotomy post-stroke functional outcomes are related specifically to the influence of estrogen on cerebral vasculature (Appelros, Stegmayr, & Terent, 2009). As such, our study includes the impact of gender on cognitive decline.

**Comorbidities.** Ischemic stroke rarely occurs in an entirely healthy patient. Rather, stroke is accompanied by a, sometimes exhaustive, list of comorbidities (often thought of as risk factors) that share a common feature—inflammation. For instance, hypertension is the leading risk factor for stroke and therefore is the most common stroke co-morbid condition. Moreover, hypertension results in systemic vascular insults that damage end organs, such as the brain, though direct and indirect methods (i.e., inflammation) (Morrison & Filosa, 2019). Other common comorbidities shared with stroke that will be investigated in this study are dyslipidemia, diabetes mellitus, arrhythmia, coronary artery disease, congestive heart failure, smoking, and major depression. Inflammation, as well as the relationship between these conditions and inflammation, will be reviewed in detail in Chapter 2.

**Assessing Comorbidity**

In this study, we used the stroke prognosis instrument (SPI-II) to tally the presence of stroke comorbidities or, comorbidity burden. When used traditionally, a diagnostic instrument
estimates the probability of recurrent stroke or death within next two years. The instrument assigns a risk group of low, medium or high based on weighted variables known for their association to cardiovascular disease and stroke. For example, congestive heart failure, diabetes mellitus and prior stroke received ‘3’ points whereas age >70 years and type of index event (stroke compared to a transient ischemic attack) received ‘2’ points, and severe hypertension (defined as a systolic blood pressure over 180mmHg or diastolic blood pressure over 100mg) and coronary artery disease received ‘1’ point (Kernan, Horwitz, Brass, Viscoli, & Taylor, 1991). In this way, the greater the SPI-II score, the greater the presence of stroke co-morbid conditions.

Mechanisms of Post-Stroke Cognitive Decline

The pathways that define a progression toward vascular dementia and the pathophysiology of post-stroke vascular dementia are poorly understood. A hypothesis that has emerged from the physiology literature and mechanistic studies is that the post-stroke inflammatory response and unresolved brain injury near the brain regions responsible for cognition is a mechanism of post-stroke vascular dementia. That many stroke comorbidities are also diseases with a prominent inflammatory component may suggest that the presence of stroke comorbidities and the occurrence of post-stroke cognitive decline may be related—a focus of the proposed research.

Vascular dementia following stroke (despite varying pathologies) is defined as a decline in brain function usually within three months after stroke onset (Pasi, Poggesi, Salvadori, & Pantoni, 2012). Cognitive decline is a very mechanistic definition that is commonly referred to in this document. Memory, thinking, language, judgment and behavior can be affected. Studies have shown that the occurrence of post-stroke cognitive decline is most prevalent in those with
large infarcts and may be related to the primary injury itself, especially when the cognitive decline occurs within the first year (Pendlebury & Rothwell, 2009). The more tissue affected, the more obvious the symptoms displayed (Kalaria, Akinyemi, & Ihara, 2016). While this appears to be straightforward, the relationship is not linear. Increasing evidence suggests that poor brain healing (known as liquefactive necrosis) occurs in the brain after stroke relative to other tissues, resulting in chronic brain inflammation and a damaged blood brain barrier (Chung et al., 2018). A damaged blood brain barrier means that the brain is no longer protected from the dangerous milieu of the systemic circulation. Chronic brain inflammation, coupled with chronic systemic inflammation (present in the form of stroke comorbidities), presents a situation that may predispose one to post-stroke cognitive decline—the focus of this study.

**Assessing Cognitive Function**

A number of cognitive screening instruments assist clinicians in early detection of mild cognitive impairment, which often progresses to more limiting dementia (Nasreddine et al., 2005). In this study, cognitive function was evaluated using two separate instruments, the Mini-Mental State Exam (MMSE) and the Montreal Cognitive Assessment (MoCA). Although discussed more thoroughly in Chapter 2, the MMSE is a 30-point screening instrument categorized by six cognitive domains. Each category is weighted based on domains responsible for cognitive aspects of mental functions (Folstein, Folstein, & McHugh, 1975). The MoCA is a 10-minute, 30-point screening instrument categorized by eight cognitive domains. (Nasreddine et al., 2005).
Study Purpose and Aims

The post-stroke inflammatory cascade when combined with brain injury are largely a hypothesized mechanism of post-stroke cognitive decline. Confounding this scenario, ischemic stroke occurs in individuals often with more than one comorbid condition that have a strong systemic inflammatory component, which we posit may increase patient susceptibility for post-stroke cognitive decline. On this basis, the purpose of this secondary data analysis is to examine the relationships between cognitive function (operationalized by the MoCA & MMSE) and stroke comorbid conditions (operationalized by the SPI-II) in male and female community-dwelling stroke survivors. The following aims organize the current study:

Aim 1

Evaluate the relationship between cognitive function and stroke comorbid conditions.

Aim 1a. Examine the association between participants’ stroke comorbidities (overall SPI-II score) and overall cognitive function (MoCA & MMSE scores).

Aim 1b. Examine the relationships between overall stroke comorbidities and the individual cognitive domains captured by the MoCA and MMSE assessments.

Aim 2

Examine the differences between overall cognitive function and individual co-morbid conditions represented in the SPI-II assessment tool.

Aim 3

Determine if post-stroke cognitive function is different according to gender in this population of community-dwelling stroke survivors.
CHAPTER 2: LITERATURE REVIEW

Inflammation

Inflammation is a general term used to describe a physiological process instigated by infection and/or injury, which triggers recruitment of initial cell responders (i.e., white blood cells/neutrophils) and proteins (i.e., cytokines/chemokines) to the affected site (Huether & McCance, 2013). Both systemic and tissue-resident macrophages—microglia—are primary players in restoring homeostatic balance to the brain by clearing debris and preparation for wound healing. Immune cell responders respond to inflammatory proteins, prompting the release of inflammatory proteins that, in turn, have a role in vascular function, specifically smooth muscle vasodilation (increased circulation & leukocyte migration) and endothelial junction permeability that allow plasma proteins and leukocytes to exit the circulation for a response in the targeted parenchyma (Newton & Dixit, 2012). In this case, the primary tissue of interest is the brain. However, inflammatory responses share some common components and responses and a chronic inflammatory or un-resolved response in any tissue results in continuous homeostatic imbalance and tissue dysfunction (Medzhitov, 2008). This concept will be discussed in relation to the brain but also, general concepts apply to the entire human organism.

Cell Responders

When activated, immune cells (neutrophils, lymphocytes, & tissue specific immune cells such as the brain’s microglia) present receptors on the surface of the host cell called pattern recognition receptors (e.g., toll-like receptors) that induce a response when pathogens or dying host cells are recognized. Pathogens are distinguishable from host-molecules by surface molecules called pathogen-associated molecular patterns (PAMPs) not typically found on host
cells. In addition to the expression of surface molecules, damage-associated molecular patterns (DAMPs) are products released by cells in response to host tissue injury, ischemia or disease conditions (Lénárt, Brough, & Dénes, 2016; Voet, Srinivasan, Lamkanfi, & van Loo, 2019). Specifically, mitochondrial DNA released from necrotic cells are recognized as DAMPs by circulating immune cells (due to their resemblance to bacteria). DAMPs activate pattern recognition receptors and cytokines, which then promote an inflammatory response to result in further tissue damage (Bajwa, Pointer, & Klegeris, 2019; Shichita, Sakaguchi, Suzuki, & Yoshimura, 2012). This activation of the innate immunity after brain injury has been implicated in post-ischemic brain inflammation and neurodegeneration related to local and systemic, oxidative damage and activation of pro-inflammatory signaling pathways (Dela Cruz & Kang, 2018; Dromparis & Michelakis, 2013; Maeda & Fadeel, 2014; Thundiyil & Lim, 2015). In more general terms, mitochondrial function and behavior are fundamental to an entire organism’s cellular health and therefore it is not surprising that mitochondrial dysfunction is associated with all of aspects of health and disease, particularly its role in chronic inflammation.

The primary functions of PAMPS and DAMPS are to recruit cells equipped to clear antibody complexes (pathogen-antigen bound neutralizing complexes), remove dead cells, and prepare the tissue/system for wound healing. It is also responsible for triggering the adaptive immune response that also contributes to the inflammatory milieu. Adaptive immune cells (T & B lymphocytes) respond to and are important in attacking non-self-pathogens in the case of infection or toxins. Adaptive immunity has two main roles, an antibody response and cell-mediated immune response. The impressive feature of adaptive immune cells is its clonal expansion of lymphocytes. Quite rapidly, there is an increase from a few cells to millions with
the original T or B lymphocyte’s antigen receptor, designed to fight the same pathogen. In an antibody response, B cells are activated to release antibodies that bind to an antigen, causing it to inactivate. Whereas T-cells directly kill infected host cells by secreting perforin, which induces holes in cell membranes or promotes ligands for death receptors (tumor necrosis factor) while also secreting pro-inflammatory cytokines (Budd, 2012; Firestein, 2013). Important to this study, the adaptive immune response (the post-stroke B-cell & T-cell responses) is implicated in antibody-antigen activation and neuroinflammation that promotes secondary neurodegeneration and post-stroke cognitive decline (Becker, Tanzi, Zierath, & Buckwalter, 2016; Ortega et al., 2015).

**Cytokines and Chemokines**

Also, as a part of inflammation, cytokines are small, low molecular weight, soluble proteins released by cells that function as chemical messengers, regulating the immune system (Becher, Spath, & Goverman, 2017). With the breakdown in the blood brain barrier that occurs after brain injury, parenchymal sources of cytokines may relocate to the systemic blood flow and visa-versa. Sources of recruited cytokines are twofold: from systemic immune cells (e.g., neutrophils, T-cells, B-cells) and tissue inflammatory cells (e.g. astrocytes & microglia in the brain) (Cheon et al., 2017). Examples of cytokines include interleukin (IL)-1ß and tumor necrosis factor (TNF) and are primarily responsible for activating innate immune cells (Newton & Dixit, 2012). Others, such as IL-6 are responsible for phenotypic shifting and differentiation of neurons and astrocytes, aiding homeostasis. Interleukin-34 regulates the growth of CNS resident microglia (Becher et al., 2017). Chemokines are a type of cytokine that are produced as a molecule signaling that attract immune cells to the injury site or source of inflammation (Becher
et al., 2017). Released in the circulation or directly into tissue, cytokines locate target immune cells and interact with the receptors on the target cell by binding to them. This triggers specific responses (good & bad) by target cells to signal for more cells to respond to the injury or disease (Loane & Kumar, 2016; Rea et al., 2018; Yang, Hawkins, Dore, & Candelario-Jalil, 2019).

**Small Molecular Messengers**

During inflammation, small molecular messengers (e.g., S100B, ROS, & NOS) are released, modulating the inflammatory response by recruiting leukocytes and inducing cytokine secretion. These molecules may also be classified as a type of DAMP. For example, the S100 family of calcium binding proteins make up almost half of cytoplasmic proteins in neutrophils and are released in larger concentrations in response to injury and disease. Responsible for accelerating the release of cytokines during inflammation, excessive expression can magnify the inflammatory response, potentially aggravating an already toxic condition (Wang, Song, Wang, Jing, & Ma, 2018). In the brain, S100B is located in astrocytes and is released with injury by these cells for local and systemic effects. Because of its small size, S100B arrives in the systemic circulation via the glymphatic system and diffusion and, at times, have been used as a biomarker of brain injury in stroke and traumatic brain injury (Plog et al., 2015). Elevated serum levels are widely considered an expression of active neural injury. S100B is correlated with severity of brain injury with higher plasma levels associated with larger infarct volumes and worse functional outcomes (Glushakova, Glushakov, Miller, Valadka, & Hayes, 2016; Michetti et al., 2019).

**Reactive oxygen species.** Another classification of molecules generated in response to inflammation are reactive oxygen species (ROS, e.g., superoxide anion) and reactive nitrogen
species (RNS). Molecular oxygen is one the primary energy staples for systemic cellular functioning and is the driving force behind oxidative phosphorylation, the process by which mitochondria produce adenosine triphosphate (ATP). This vital function is connected to the creation of highly reactive and toxic side products, reactive oxygen species (ROS), and is produced in high amounts by NADPH oxidase complexes (NOX). The most potent of these complexes is NOX2, predominantly expressed by phagocytic cells, and is the dominant ROS-producing complex in mammals. While ROS production by phagocytes occurs during the oxidative burst and is essential for killing pathogens, it is also implicated in promoting inflammation and tissue damage (Hoffmann & Griffiths, 2018; Murphy, 2009). Therefore, cellular ROS formed as a result of mitochondrial oxidative phosphorylation—a constitutive intracellular, energy producing process—when dysregulated, disrupt cellular respiration (Murphy, 2009). Because of a partial reduction of oxygen, negatively charged ion particles increase, overwhelming the antioxidant defense system. Persistent oxidative stress, either direct or indirect, are implicated in the pathogenesis of chronic diseases at both the microcellular and macrocellular states. For example, reactive oxygen species regulate several inflammatory signaling pathways (Ray, Huang, & Tsuji, 2012).

Nitric oxide synthases (NOS) is a catalyst enzyme found in macrophages and is responsible for producing nitric oxide, a type of RNS and one of the smallest signaling molecules (Förstermann & Sessa, 2012). Constitutively expressed in small concentrations in the healthy brain, nitric oxide serves as a neurotransmitter, maintains vascular tone, and has a key role in DNA/RNA transcription and translation. However, in abundance and in its inactivated state (secondary to reacting with ROS), oxidant peroxynitrite (ONOO⁻) causes oxidative damage to
proteins, lipids and DNA. Inducible NOS, when triggered by bacterial components and cytokines, produces large amounts of nitric oxide in an immune response, undesirable to pathogens. As such, RNS, produced by an abundance of activated macrophages and neutrophils can contribute to tissue damage known as secondary damage (discussed below). Inflammatory neurodegeneration is one example where an overabundance of neuronal NOS derived nitric oxide induces neuronal death (Förstermann & Sessa, 2012; Ray et al., 2012).

**Cell Signaling in Inflammation**

This brief overview of some of the molecular players in inflammation illustrate that multiple pathways are responsible for cell signaling to promote a defense against invading pathogens that also initiate, compound, or imbalance, to mute inflammatory states. Some, as in the case of toll-like receptors, can concurrently activate multiple proinflammatory cell signaling pathways. Toll-like receptors are part of a family of pattern recognition receptors that specifically recognize PAMPs and play a vital role in innate immunity. Activated pattern recognition receptors are part of the first line of defense, initiating signaling cascades that release factors that promote, for example, neutrophil recruitment to the site of infection or injury. They are critical in immune cell regulation, inflammation and survival (Newton & Dixit, 2012).

Moreover, enzyme complexes and proteins (i.e., inhibitor of nuclear factor kappa B kinase [IKK] enzyme complex & TAK1 proteins) become activated by toll-like receptor cascades that then activate the nuclear factor kappa light chain enhancer of activated B cells (NFkB) pathway—a prominent transcription factor related to the inflammatory response. As a result this activation, NFkB may induce genes that either propagate or inhibit the inflammatory
response, to limit further tissue damage, and therefore, is an important player in inflammatory responses to balance the “good” and “bad” of such responses (Newton & Dixit, 2012).

Another prominent inflammation signaling cascade that occurs in tissue, including the brain, is carried out by inflammasomes. Inflammasomes are complex, multiprotein, intracellular sensors of PAMPs and DAMPs. Immune cells, such as microglia in the brain, release pro-inflammatory cytokines when responding to injury, specifically IL-1β & IL-18 that then initiate the inflammasome response. Part of this response may result in pyroptosis, an inflammatory form of programmed cell death that is implicated in a number of neuropathologies (Freeman & Ting, 2016; Voet et al., 2019). These examples illuminate the broad network of a response that encompasses inflammation. Once triggered, it is difficult to bring into balance or silence, perpetuating a chronic aspect of disease and injury.

**Ischemic Stroke**

The brain is subject to the inflammatory responses described above, particularly in chronic disease and injury. It is the most metabolically active organ in the human body and depends on constant blood flow to supply the metabolic needs for basic brain cell functioning (Cipolla, 2009). Ischemic stroke occurs when a blood vessel in the brain becomes occluded, because of either thrombus or emboli. Ischemia results when blood flow is disrupted for less than 10 minutes or cerebral blood flow falls to less than 12mls/100g brain tissue/min (Mohr et al., 1997). In less than 40 seconds of complete global ischemia, there is complete cessation of electrical activity (Dreier et al., 2018; Morrison & Filosa, 2019). Primary injury is the result of ischemia while secondary injury involves cell death mechanisms, not associated with nutrient deprivation but instead, those associated with inflammatory mechanisms (Zille et al., 2012).
Primary Injury

Acute cessation of cerebral blood flow produces an almost immediate energy failure to surrounding brain tissue. The focal impairment of vital nutrients, primarily oxygen and glucose, disrupt the process of oxidative phosphorylation—the process that powers the mechanisms underlying brain information processing (Hall, Klein-Flugge, Howarth, & Attwell, 2012). Glutamate and gamma-aminobutyric acid (GABA) are the primary neurotransmitters responsible for excitatory and inhibitory mechanisms of synaptic transmission in the healthy brain. However, with ischemia and resulting energy failure, glutamate and GABA are dysregulated because transporters are not maintained. Excitotoxicity results, an injury mechanism where there is an excessive release of glutamate that leads to over-activation of post-glutamate receptors and neuronal dysfunction (Trendelenburg & Dirnagl, 2005). In addition, GABA receptor response is reduced, impairing inhibitory mechanisms that further potentiate neuronal insult (Mayor & Tymianski, 2017; Rose et al., 2017). Persistent injury and excitotoxicity further reduce ATP concentrations, increases intracellular calcium concentrations, resulting in mitochondrial dysfunction and, eventually necrosis (Chauhan, Moser, & McCullough, 2017; Mayor & Tymianski, 2017). Particularly harmful, necrosis occurs rather than apoptosis where cells burst (rather than programed cell death) and release intracellular contents, to include mitochondria, that potentiate secondary injury mediated by DAMPS (discussed above) (Borgens & Liu-Snyder, 2012).

Secondary Injury

Minutes after the initial ischemic injury, intracellular and extracellular biochemical processes characterize secondary injury and occur concurrently with primary injury. This stage
refers to the wave of molecular events that trigger the death of “healthy” neighboring cells in the penumbra—also called the peri-infarct region. The penumbra is the immediate, metabolically salvageable tissue, which is hypoperfused brain tissue surrounding the ischemic core (del Zoppo, Sharp, Heiss, & Albers, 2011; Goyal et al., 2016). Secondary injury occurs primarily within the penumbra and sources include reperfusion injury, neuroinflammation, edema (cell & vascular), and systemic inflammation. Parenchyma edema, induced by primarily cytotoxic factors, increases blood-brain barrier permeability and neuronal swelling to result in additional necrosis and apoptosis (Borgens & Liu-Snyder, 2012).

Reperfusion occurs when blood is returned to the tissue after a period of ischemia. Reperfusion injury is a secondary injury that compounds inflammatory responses. For example, neutrophil recruitment—a white blood cell in the blood—is exaggerated and there is transmigration of neutrophils across the blood-brain barrier. Neutrophils enter the parenchyma, release proteases and exert oxidative stress on injured neurons in the penumbra (Enzmann, Kargaran, & Engelhardt, 2018). Reperfusion also activates the systemic inflammatory response and potentiates the “no-flow phenomenon” initiating additional inflammatory cascades resulting in injury. The “no-flow” phenomenon occurs when, despite the re-establishment of blood flow, blood flow is impeded due to vascular and blood changes resulting from mechanisms of primary and reperfusion injury. For example, after primary injury and with reperfusion, blood increases in viscosity, red blood cells aggregate with themselves and neutrophils. Neutrophils then bind to the endothelium where precapillary shunting and vascular constriction also occur. Combined, these phenomena narrow the vasculature and increase cell accumulation to block blood flow in the smaller brain vessels known as the microvasculature (Fischer, Ames, Hedley-Whyte, &
O'Gorman, 1977). Reperfusion injury extends stroke volume beyond the ischemic injury, and the degree of this reperfusion injury is directly associated with the severity of the primary injury. Although reperfusion is necessary to stop the primary injury caused by ischemia, the return of oxygen, nutrients and immune cells start a wave of biochemical processes that are damaging to the penumbra. There lies the dilemma whereby resolution is impossible to attain. With reperfusion, a secondary mechanism of injury is initiated, yet without reperfusion, ischemia persists, and serious disability or death will occur.

Blood-Brain Barrier Dysfunction

During the period of cerebral ischemia, injury significantly compromises the blood-brain barrier’s structural integrity. The blood-brain barrier is composed of endothelial cells, astrocyte endfeet, pericytes, and a basement membrane made from structural proteins such as the extracellular matrix proteins, collagen and laminin. This semi-permeable structure regulates the entry of solutes, molecules, and cells from the vasculature and the removal of systemic toxins. Through coordinated efforts, these cellular components form the overall outcome of preventing vascular leakage from the systemic circulation to the parenchyma and vice versa (Rhea & Banks, 2019). During prolonged neuroinflammation, damage to cells caused by primary and secondary brain injury impair the physical and functional blood-brain barrier (Haruwaka et al., 2019). Profound and/or chronic hypoperfusion can induce the production of vasodilatory substances such as carbon dioxide and nitric oxide that cause dysfunction of the endothelium. Persistent ischemia-induced inflammation eventually impacts the efficacy of the barrier function (Beridze, Sanikidze, Shakarishvili, Intskirveli, & Bornstein, 2011). In response to ischemia, circulating neutrophils in the vasculature quickly localize in an abundance proportional to the severity of
infarct, responsible for a number of neutrophil-mediated defense mechanisms (Jickling et al., 2015; Ritter, Orozco, Coull, McDonagh, & Rosenblum, 2000; Rosales, Lowell, Schnoor, & Uribe-Querol, 2017).

Astrocytes are brain glial cells responsible for maintaining a chemical environment for brain cell signaling (Purves, 2008). Part of the neurovascular unit, astrocytes’ endfeet link blood vessels to neuronal circuitry by wrapping around the vasculature and cell bodies (pericytes), which are in close proximity to neurons. Astrocytes also have an inflammatory function. During an acute brain injury, astrocytes activate a host of immune mediators that break down the blood brain barrier and allow the entry of additional immunomodulatory cytokines. In addition, their endfeet become removed from the vasculature (Filosa, Morrison, Iddings, Du, & Kim, 2016). A dysfunctional blood-brain barrier can lead to dysregulation of ions, altered signaling, entry of immune cells and molecules into the central nervous system (or “leakiness”), leading to neuronal dysfunction and degeneration (Daneman & Prat, 2015). After approximately three days, bleeding and leakiness start to subside forming a glial scar (Kumosa, Zetterberg, & Schouenborg, 2018). Neural wound healing and cellular stabilization occur within six weeks (Burda & Sofroniew, 2014). While blood-brain barrier dysfunction is typically seen as a result of an acute injury, in some cases, chronic inflammation or injury related to a disease can break down this barrier (Daneman & Prat, 2015).

Immune cell responders. The process of neuronal injury involves various intracellular mechanisms. Initial cell responders include systemic circulating innate immune cells that include white blood cells (i.e., neutrophils, basophils & eosinophils), macrophages, dendritic cells (i.e., antigen-presenting cells) mast cells and resident immune cells. In this case, the primary, resident
immune responders central to the brain are microglia and astrocytes. Systemic neutrophils and macrophages as well as brain microglia are phagocytic cells with the ability to ingest invading pathogens. Of all the cell types, much focus has been on how phagocytes contribute to post-stroke brain injury and resolution, to include post-stroke cognitive impairment and, in general, chronic disease (Winterbourn, Kettle, & Hampton, 2016).

Microglia, as the brain’s resident phagocyte, have been scrutinized for their beneficial versus harmful role in neuroinflammation, post-stroke brain injury and cognitive decline (Doyle & Buckwalter, 2012; Nguyen et al., 2016). Microglia represent approximately 10% of total central nervous system (CNS) glial cells (Loane & Kumar, 2016). Microglia help maintain synaptic signaling, acting as scaffolding for signal propagation, neural development and aiding recovery from neural injury. These cells are derived from hematopoietic cells and neural stem cells, functioning like neural scavenger cells. They can be recruited from resident brain areas but also from the circulation (Purves, 2008; Sokolowski & Mandell, 2011). They act as phenotypic conductors, orchestrating an array of inflammatory molecules (Loane & Kumar, 2016). At the point of brain injury, phenotypic alteration occurs—macrophage polarization—that express PAMPS and produce DAMPs in response to injury and disease. Concurrently, this hypoxia/ischemia continues to trigger adenosine triphosphate (ATP) and DAMPs that stimulate toll-like receptors responsible for recruiting more astrocytes and microglia to the site of injury (Amantea, 2016; Mayor & Tymianski, 2017). When this occurs, microglia acquire an amoeboid morphology, which collect and discard metabolic and tissue debris in the healthy brain. However, when cerebral ischemia occurs, macrophages and resident microglia activate and migrate to the site of injury, changing their morphology between M1 phenotypes, which promote
a pro-inflammatory response and M2 phenotypes that demonstrate anti-inflammatory properties, preventing further activation of neuroinflammatory mediators and tissue damage (Cheon et al., 2017).

Additionally, following an ischemic stroke, the bone marrow is also stimulated, increasing production of available monocytes that are recruited to the ischemic core with a higher phagocytic capacity than resident microglia. At 90 minutes, microglia taper while circulating monocytes are thought to assume area phagocytic responsibilities. This recruitment peaks at approximately 72 hours (Ritzel et al., 2015).

**Measuring Brain Injury after Ischemic Stroke**

Multiple tools are available to measure stroke severity. Historically, computed tomography has been the primary stroke assessment tool to quantify stroke volume (stroke size) (Leiva-Salinas, Jiang, & Wintermark, 2018). Volume of infarcted tissue alone has only been moderately correlated with stroke outcomes; however, integrating stroke lesion size and location with stroke assessment tools have further allowed clinicians to more accurately predict functional recovery over time.

A variety of stroke scales that quantify motor and cognitive function additionally aid to improve diagnosis, suitability for specific treatments, monitor change in neurologic impairments, and further predict outcomes. For example, by correlating ischemic brain areas with severity of modified Rankin scores (mRS), stroke-related disability was predicted. Ischemic brain regions more centrally located (e.g., caudate nucleus, putamen & hippocampus) were of high mRS relevance (equivalent to poorer functional recovery) whereas areas of low mRS relevance were more peripherally located and include the temporal, frontal and occipital gyrus (Ernst et al.,
The mRS measure functional independence (specifically an ability to walk with or without assistance) on a seven graded scale (Appendix C) (van Swieten, Koudstaal, Visser, Schouten, & van Gijn, 1988).

The National Institute of Health Stroke Scale (NIHSS) is a dominant stroke impairment scale in use following an acute event. It is a 15-item scale that has been shown to have long-term predictive outcomes (Appendix C) (Adams et al., 1999; Brott et al., 1989). Infarct volume has been correlated with scale scores and when including the peri-necrotic region, associations were strengthened (Alexander et al., 2010). Finally, another measure of disability is the Barthel Index (BI), which measures self-care and physical dependency. The Barthel Index is the one of the most common functional assessment scales in stroke, primarily in adult rehabilitation, demonstrating prognostic utility in clinical practice. Stroke patients with an initial score of 60 were associated with shorter lengths of stay while those under 40 lost independence in mobility skills, needing long-term assistance (Appendix C) (Granger, Dewis, Peters, Sherwood, & Barrett, 1979; MacIsaac et al., 2017).

Stroke dysfunction moves beyond just motor functions but also includes cognitive and mental dysfunctions to include cognitive impairment, vascular dementia, psychological symptoms and post-stroke fatigue. Nearly 70% of patients within two months following stroke describe at least one symptom of cognitive impairment. Because of disrupted circulation to specific areas of the brain responsible for executive functions, vascular dementia impairs cognitive abilities (Khan, Kalaria, Corbett, & Ballard, 2016). Further, the level of cognitive impairment was significantly associated with depressive symptoms, anxiety and all psychological factors (Nijssse et al., 2017). Confirming these findings, Macintosh et al. (2017)
found cognitive impairment was also significantly associated with depressive symptoms. These same authors also noted an association between post-stroke fatigue and impaired mobility (MacIntosh et al., 2017). Although there appears to be overlapping effects on functional outcomes, fatigue alone also needs emphasis given its impact on post-stroke recovery.

Complicating post-stroke recovery are the visual impairments, which are often correlated with more severe strokes, poorer quality of life measures and increased disability (Sand et al., 2016).

**Inflammation: The Common Denominator of Ischemic Stroke, Cardiovascular-Related Diseases and Post-Stroke Cognitive Decline**

In this study, our goal is to study the relationship between the presence of cardiovascular related diseases that are common co-morbid conditions to stroke and post-stroke cognitive function in community dwelling stroke survivors. In the preceding sections, we have focused on understanding the intricacies of inflammation and stroke injury mechanisms. In this section, this discussion is expanded to describe the role of inflammation in cardiovascular-related diseases (to include smoking) and depression that then creates a chronic systemic inflammatory status that may influence post-stroke cognitive function. These relationships are illustrated in Figure 2.

Inflammation can lead to vascular changes and activation of innate immune cells as discussed in previous sections above (Lénárt et al., 2016). Cellular injury attributable to systemic inflammation can manifest itself through endothelial dysfunction affecting its ability to perform its many roles and may result in declining cognitive function, independent of stroke (Iadecola & Gottesman, 2019). In addition, the chronic presence of systemic inflammatory stimuli due to chronic cardiovascular-related conditions such as smoking, hypertension, dyslipidemia and diabetes (detailed in the next sections) may negatively impact the post-stroke brain that is no
longer protected by an intact blood brain barrier. It must further be emphasized that when multiple inflammatory diseases overlap, deleterious results ensue and may directly or indirectly influence post stroke cognitive outcomes. Inflammation, then, becomes a common thread that both influences disease risk, disease progression, and disease recovery. This common thread—inflammation—will be discussed as it relates to the influence of stroke co-morbidities common in post-stroke patients demonstrating cognitive decline.

**Cardiovascular-Related Comorbidities**

**Hypertension.** Hypertension is defined as having a systolic blood pressure equal or greater than 140mmHg (Benjamin et al., 2018). The etiology of hypertension is multifactorial; it is a complex interaction between both cardiovascular and central nervous system mechanics (Biancardi, Bomfim, Reis, Al-Gassimi, & Nunes, 2017). There is now an accumulation of evidence that suggests that altered immunity and inflammation are also important contributors to the development of hypertension. It is well established that dysregulation of the renin-angiotensin system (RAS) causes hypertension directly through its effects on the kidneys, blood vessels and central nervous system (Paul, Poyan Mehr, & Kreutz, 2006). More recently, studies have implicated adaptive immune responses, specifically lymphocytes as playing an inflammatory role in hypertension (McCarthy et al., 2014; McMaster, Kirabo, Madhur, & Harrison, 2015). The innate immune system (responsible for activating the adaptive immune response) relies heavily on toll like receptors to initiate downstream signaling that triggers an inflammatory response (Bomfim et al., 2012). Of the TLRs, TLR4 is well recognized in its etiology of hypertension and specifically induces activation of the NF-kB pathway (Akira & Takeda, 2004). Toll-like receptor-4 mediated signaling, specifically on brain microglia, increase
inflammatory and oxidative states that have been shown to coincide with the development of hypertension (Wu, Chan, & Chan, 2012).

Pivotal to the current study, the cerebral vasculature is the primary structure most affected by the deleterious effects of hypertension or chronically elevated blood pressure. Vasoconstrictive, maladaptive remodeling occurs decreasing lumen diameter and increasing intima thickness. Vascular inflammation, immune cell infiltration and endothelial dysfunction lead to lumen stiffness, eventually causing hypoperfusion to the neurovascular unit (neuron, astrocytes and vascular cells) that may directly lead to cognitive impairment (Iadecola & Gottesman, 2019).

**Congestive heart failure.** Congestive heart failure (CHF) is generally recognized as a myocardial disease that affects cardiac contractility and reduced left ventricular function (Yancy et al., 2017). It is typically a chronic, progressive heart disease as a result of lost or impaired cardiac muscle (Cohn, Ferrari, & Sharpe, 2000). Inflammation is a key pathological mechanism associated with neurodegeneration in the setting heart failure (Hatanaka et al., 2015; Xu & Li, 2015). Previously, left ventricular systolic dysfunction was implicated as the primary pathomechanism involved in vascular-related dementia. Studies now support, independent of brain hypoperfusion, progressive inflammation of the brain endothelium leads to dysfunction of the blood brain barrier, resulting in vascular cognitive impairment (Adamski et al., 2018).

Congestive heart failure is well known for its secondary impairment on other organ systems. In this aging population, neurocognitive impairment is high (specifically termed cardiogenic dementia) and is a common co-existing condition among those affected by heart failure (Agüero-Torres, Thomas, Winblad, & Fratiglioni, 2002). There is consistent evidence that
neurocognitive impairment progresses as the disease evolves; in fact, individuals with mild to moderate heart failure were four times more likely to have cognitive impairment than controls (Frey et al., 2018).

**Diabetes mellitus.** Diabetes mellitus is a condition defined by high blood glucose levels due to either inadequate insulin secretion or insulin resistance (Sabertzadeh-Ardestani et al., 2018). Studies have linked higher blood sugars with cognitive decline compared to those without diabetes, specifically slower information processing speed and memory recall (Palacios-Mendoza et al., 2018). Insulin resistance has been shown to be responsible for decreased glucose metabolism in the brain, a potential cause for decreased neurogenesis and increased neuronal atrophy in the diabetic brain (Hamed, 2017). Other proposed mechanisms include mitochondrial dysfunction, which induces pro-inflammatory cytokines that pass through a leaky blood brain barrier contributing to neuroinflammation in the setting of endothelial dysfunction (Hsieh, Liu, Lee, Yu, & Wang, 2019). Moreover, acute alterations in glucose further impair brain repair processes (Hamed, 2017; Plog & Nedergaard, 2018).

**Coronary artery disease and dyslipidemia.** Coronary artery disease is type of heart disease where arteries that supply blood to the heart become diseased or damaged, affecting blood flow. This is often related to dyslipidemia, a disorder associated with an abnormal level of lipids found in the blood (Oliveira, Bellozi, Reis, & de Oliveira, 2018). While lipids are vital for many functions (e.g., cell signaling, cell-structure & myelination), if in excess, has been linked to a number of vascular-related diseases. Libby and Ridker (1999) and Ridker et al. (2000) specifically reported that increased TNF and IL-6, respectively, were present among patients
with ischemic heart disease and correlated highly with those who were high risk for future attacks (Libby & Ridker, 1999; Ridker et al., 2000).

During the response to myocardial injury, platelet aggregation is responsible for releasing a potent mitogen called platelet-derived growth factor (PDGF). PDGF stimulates the production of “hyaluronic acid, proteoglycans and collagen” that lay the foundation for extracellular matrix and vascular smooth muscle cell deposition (Newby, 2000). In an attempt to restore vascular integrity, smooth muscle cells phenotypically switch their pattern of gene expression to one that favors migration, division, and synthesis of interstitial matrix components (i.e., fibronectin, collagen type I & elastin) (Thyberg, 1998). Continued deposition of fibrous tissue characterizes an advanced lesion by its fibrous cap perpetuating apoptosis (Huether & McCance, 2013; Stoneman & Bennett, 2004). Inflammatory cytokines within the lesion also regulate smooth muscle cells proliferation and apoptosis that contribute to the continued cascade of effects and a continuation of systemic inflammation. For example, vessel wall cells oxidize low-density lipoprotein (LDL) that release cytokines/chemokines to signal the cell responders such as monocytes (Ross, 1993). Monocytes migrate beneath the arterial surface at endothelial cell junctions, becoming macrophages, ingesting lipids and forming foam cells that progress to fatty streaks that are the underlying cause of atherosclerosis (Ross, 1993). Newby (2000) reported “the ability of oxidized-LDL to promote both endothelial adhesion molecule expression and production of chemokines such as monocyte chemotactic peptide-1 (MCP-1) and smooth muscle cells may be a key initiator of the inflammatory response during atherosclerosis” (Newby, 2000). More recently, Kapsimalis et al. (2008) state these underlying mechanisms are triggered by TNF and IL-6 (Kapsimalis et al., 2008). Early macrophage-foam cells further drive the progression of
atherosclerosis by inherently secreting pro-inflammatory cytokines and growth factors responsible for smooth muscle cell proliferation but also drive systemic inflammation (Packard & Libby, 2008).

**Arrhythmias.** Atrial fibrillation is a common cardiac arrhythmia and is often associated with heart failure and stroke. Worldwide, more than 30 million are affected and that number continues to grow (Chugh, Roth, Gillum, & Mensah, 2014; Naccarelli, Varker, Lin, & Schulman, 2009). Atrial fibrillation is primarily caused by both electrical and structural remodeling of the atria, affecting the heart’s ability to regulate electrical signals. During atrial remodeling, underlying mechanisms of systemic inflammation are evident. While a number of cardiovascular comorbidities promote inflammation, atrial fibrillation independently stimulates the production of pro-inflammatory cytokines (IL-6, C-reactive protein & TNF) that activate a number of immune cells (neutrophils, macrophages and mast cells) (Frangogiannis, 2014; Yamashita et al., 2010). Primary treatment options include heart rate control and/or rhythm control (a return to normal sinus rhythm) to prevent cardiovascular consequences as described earlier (Hu, Chen, Lin, & Chen, 2015).

**Major depression.** Depression is a mental health disorder characterized by behaviors of apathy, lack of motivation or excitement, inter-personal problems and reduced productivity (Sachdev, 2018). More than 5% of the world’s population is affected by depression and it is the leading cause of death and disability among 15 to 29 year olds (WHO, 2017.). There is a substantial body of knowledge that depression and inflammation are interrelated. A presence of pro-inflammatory cytokines and immune cells have been associated with the onset of depressive-like symptoms and those with clinical depression have demonstrated consistently higher markers
of inflammation (Dowlati et al., 2010; Liu, Ho, & Mak, 2012). Inflammatory markers are related to decreased tryptophan, a precursor to the neurotransmitter—serotonin—and reduces neurotransmitter metabolism in the brain (Maes, Leonard, Myint, Kubera, & Verkerk, 2011). Further, a migration of peripheral immune cells into the brain in response to stress coincides with activation of microglia and pro-inflammatory cytokines throughout various brain areas, specifically associated to mood disorders (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Wohleb, McKim, Sheridan, & Godbout, 2014).

As it relates to this study, a number of comorbidities have demonstrated injury mechanisms that overlap with neurodegenerative mechanisms, including oxidative stress, mitochondrial dysfunction and progressive neuroinflammation (Pugazhenthi, Qin, & Reddy, 2017). Overtime, these diffuse and subtle degenerative mechanisms may result in or augment post-stroke cognitive impairment and dementia.

**Post-Stroke Cognitive Decline**

Mild cognitive impairment or vascular dementia is defined as the development of cognitive decline following stroke (Brainin et al., 2015). It is estimated that approximately 10% of stroke patients will already have dementia prior to stroke, 10% more will have dementia following stroke and, 30% will go on to develop cognitive impairment with stroke reoccurrence (Pendlebury & Rothwell, 2009). Post-stroke cognitive impairment can occur immediately following stroke and due to brain cell necrosis following ischemic injury (Hénon, Pasquier, & Leys, 2006) or may present days to months later, and more specifically related to post-stroke inflammation and incomplete wound repair (characterized by a leaky glial scar) (Doyle & Buckwalter, 2020; Doyle, Quach, Solé et al., 2015; Nguyen et al., 2016; Zbesko et al., 2018).
Clinical studies have associated the neuroinflammatory response as largely responsible for cognitive decline (Thiel & Heiss, 2011; Whitehead, Cheng, Hachinski, & Cechetto, 2007). Wound healing in the brain is a unique process and differs from other organ systems, for example, the heart (Chung et al., 2018). At the site of the infarcted tissue’s ischemic core is degradation of brain tissue, which transforms into a liquefactive mass. This liquefactive mass, in response to injury, is composed of inflammatory cells at the infarcted tissue’s ischemic core. This mass is, ideally, effectively walled off by the brain’s immune cells (astrocytes & microglia) forming a glial scar, however, recent research in rodent models suggest that this is not the case (Chung et al., 2018; Zbesko et al., 2020; Zbesko et al., 2018). In contrast, the glial scar is permeable to inflammatory products (reviewed in the sections above) such that inflammation from the brain is transferred to the systemic circulation and systemic inflammation passes the glial scar to impact the nearby parenchyma and related brain functions, such as cognition (Doyle, Quach, Sole, et al., 2015; Zbesko et al., 2020; Zbesko et al., 2018).

Figure 2 summarized the totality of the sections above as well as this information. People who have strokes often have co-morbid conditions—a comorbidity burden—that increases systemic inflammation, visualized in left side of the figure. On the right, we illustrate that stroke itself contributes to brain and systemic inflammation, a result of poorly resolved brain injury with a leaky glial scar that is far inferior to the robust blood brain barrier, when intact. Therefore, post-stroke cognitive decline may be influenced not only by an ischemic stroke itself, but also by the inflammatory products and milieu of the patient with multiple co-morbid conditions. As such, in this study we aim to investigate the relationships between post-stroke cognition and
patient co-morbid conditions among community dwelling and participants that have recovered from ischemic stroke.

**Figure 2**

*The Influence of Pre-stroke Comorbidity Burden on Post-Stroke Cognitive Decline*

There are a number of cognitive assessment tools available. With the world’s aging population, the prevalence of cognitive disorders is growing, affecting nearly 50 million people (Prince et al., 2013). Therefore, cognitive screening tools are routinely used to discern between the cognitive decline of healthy aging and neurodegenerative diseases. Despite the variety of cognitive assessment tools, there is no consensus on choice of instrument to use, in part due to the multitude of factors affecting accurate outcome measures (timing of evaluation after stroke, assessment setting, baseline education, length of test, & test administrator) (Brainin et al., 2015).
Two common cognitive assessment tools are the Montreal cognitive assessment (MoCA) and the Mini mental state examination (MMSE) (Appendix D).

**Montreal Cognitive Assessment**

The MoCA is a 30-point screening instrument categorized by eight cognitive domains intended to be completed in 10 minutes. Each category is assigned higher points based on items that originally discriminated well with mild cognitive impairment (visuospatial/executive=5, naming=3, memory=0, attention=6, language=3, abstraction=2, delayed recall=5 and orientation=6) (Nasreddine et al., 2005). The range for this instrument is 0 to 30. A score of 25 or less indicates need for a referral for cognitive evaluation.

While intuitively designed, iterative adaptations of the original instrument led to an easy, more rapid tool that discriminated well between Alzheimer’s disease, mild cognitive impairment and normal cognition among elderly individuals. Developed in 2005, the MoCA demonstrated excellent sensitivity in differentiating between mild cognitive impairment and Alzheimer’s disease when compared to the MMSE (Nasreddine et al., 2005). The authors explain the MoCA’s superiority is related to memory testing that involved more words, fewer learning trials and longer delays before initiating recall, a key component in cognitive impairment. In the original study to establish the use and validity of this assessment tool, the sample population consisted primarily of elderly (>70 years old) male and female Canadian participants (N=94) with mild cognitive impairment, Alzheimer’s disease and healthy controls. This instrument was reported to have high internal consistency (Cronbach’s α = 0.83) and high test-retest reliability (r = 0.92).
Mini Mental State Examination

The MMSE is a 30-point screening instrument categorized by seven cognitive domains, eleven individual tasks. Each category is weighted based on domains responsible for cognitive aspects of mental functions (orientation=10, registration=3, attention/calculation=5, recall=3, language=2, repetition=1, and a range of complex commands =6)(Folstein et al., 1975). The range for this instrument is 0 to 30. Further evaluation is recommended for scores less than 25. Intended to differentiate psychiatric patients from those with organic dementia, the MMSE has been used extensively in Alzheimer’s, Parkinson’s and age-related cognitive decline. Repeated administrations have been used to differentiate between normal age-related cognitive decline and neurodegenerative pathological processes. Among a Canadian community sample of mixed medical patients, the Cronbach’s alpha ranged from 0.65 to as high as 0.96 (Tombaugh, 2005).

Comparing the two instruments, others have determined that the MMSE and MoCA are highly correlated (r = 0.87) in detecting mild cognitive impairment (Nasreddine et al., 2005; Shen et al., 2016). Between MMSE and MoCA, comparison studies demonstrated similar AUC (discriminatory ability), sensitivity, and specificity in detecting post-stroke cognitive impairment, regardless of age and education (Dong et al., 2014; Dong et al., 2013; Shen et al., 2016). Since the MMSE has been scrutinized for not detecting early dementia, the MoCA was developed to better identify the population of individuals that meet criteria for mild cognitive impairment yet score in the “normal elderly” range (>26). As a result, the MoCA instrument has been translated into numerous languages and demonstrated its reliability across different populations (e.g., China, Japan) in detecting mild cognitive impairment (Cronbach’s α>0.75) (Aguilar-Navarro et al., 2018; Iiboshi et al., 2019; Li et al., 2018; Wong et al., 2018).
Gender Differences

We include gender as an important biological variable in research brain research (McCarthy, Arnold, Ball, Blaustein, & De Vries, 2012; Zagni, Simoni, & Colombo, 2016). Stroke incidence appears to be greater for women starting after 71 compared to 68 in men (Appelros et al., 2009; Madsen et al., 2017). From a clinical standpoint, a potential cause is that women may be less likely to reach therapeutic blood pressure targets (Aronow et al., 2011; Lloyd-Jones, Evans, & Levy, 2005). Risk factor control may also be different between women and men as in the case with diabetes (Peters, Huxley, & Woodward, 2014). Much of the research in this area relies heavily on self-reporting of risk factors and many instruments do not likely capture the more unique risk factors specific to women such as the presence of atrial fibrillation (Madsen et al., 2017).

Sex hormones in women are primarily made up of estrogen but also include progesterone, follicle stimulating hormone and luteinizing hormone. The latter two, are produced by the pituitary gland and regulate the secretion of estrogen and progesterone. Estrogens influence important aspects of healthy brain biochemistry and morphology important for cognition. Specifically, estrogens do this by directly influencing the abundance of neurotransmitters responsible for neuronal growth and the formation or remodeling of synapses (McEwen, 2001). Estrogen also plays a role in cerebral vasculature. Estrogen has a vasodilation effect and lowers endothelium reactivity, also contributing to its neuroprotective effects (Samai & Martin-Schild, 2015).

Sex differences are also seen in microglia’s response to injury—the brain’s primary immune cell and inflammatory responder. Microglia are sexually dimorphic phagocytes
distributed throughout the brain prior to birth. Sex differentiation occurs early in brain development with differences noted in both regional volume and microglia morphology (Lenz, Nugent, Haliyur, & McCarthy, 2013). When comparing male and female mice after middle cerebral artery (MCA) occlusion, microglia volume and morphology were changed in brain regions after ischemia. Photomicrograph comparisons revealed differences between microglia process endpoints per cell immediately after MCA occlusion according in proximity to the brain lesion (Morrison & Filosa, 2016). Additionally, sex differences were observed in microglia phagocytosis. Microglia CD11b, an integrin receptor key, is higher in female mice compared to males. After ischemia, females remained high or unchanged; however, males are increased from baseline. This phenomenon suggests that female microglia at baseline have an enhanced phagocytic ability with the innate ability to engulf cell contents otherwise capable of worsening neurotoxicity (Morrison & Filosa, 2016). While similar findings reporting estrogen’s neuroprotective effects in reducing infarct size have been demonstrated, microglia phagocytosis phenotype shifts are novel (Carswell, Dominiczak, & Macrae, 2000).

Ischemic stroke is considered a sexually dimorphic disease with females at a lower incidence to males in advancing age. It is now well understood that neurocellular metabolism and immune-mediated signaling varies between males and females. While the exact mechanisms are unknown, animal studies have demonstrated smaller infarct sizes in young female mice in comparison to age matched male mice (Cheng & Hurn, 2010; Manwani et al., 2015). However, this neuroprotective (Strimbu & Tavel, 2010) effect dissipates with aging or ovarectomized female mice (Clevenger et al., 2018; Manwani et al., 2015; Morrison & Filosa, 2016). It is
hypothesized that these neuroprotective mechanisms are a result of a number of factors, and among them, brain and systemic inflammatory responses.
CHAPTER 3: METHODS

Study Sample

In the original study conducted by Taylor-Piliae et al. (2014), 145 participants were recruited over a three-year period (January 2009 to January 2012) to participate in an intervention study to evaluate the effect of Tai Chi on physical function, fall rates and quality of life among community dwelling stroke survivors (Taylor-Piliae, Hoke et al., 2014). In the current study, baseline data collected from this primary research were used for a secondary data analysis. In the current study, 97 participants were retained from the original study’s data; 48 participants were excluded because baseline data collection did not initially include cognitive assessments (both MoCA & MMSE).

Participant Recruitment

In the original study, participants were recruited from Pima County, Arizona using multiple recruitment strategies to include news media (e.g., newspaper, radio & television), medical offices, outpatient rehabilitation centers and community fitness centers. A total of 393 individuals demonstrated initial interest. Study staff based on inclusion and exclusion criteria determined eligibility. Less than half of interested persons (approximately 40%) were not candidates due to exclusion and inclusion criteria (Taylor-Piliae, Boros, & Coull, 2014).

Inclusion criteria. Male and female participants were enrolled in the study; no ethnicity was excluded (Taylor-Piliae et al., 2014). Inclusion criteria included those persons that were willing to participate in study randomization, willing to regularly participate in Tai Chi classes, and over the age of 50 who experienced a stroke more than three months prior to study (Taylor-Piliae, Boros et al., 2014).
Exclusion criteria. Exclusion criteria included those with no prior stroke, lack of time or lost interest, refused randomization, age less than 50, geographical move, previously practiced Tai Chi or SilverSneakers program, MMSE score < 18, transportation issues or a stroke that occurred less than three months prior to study enrollment. Other reasons included severe disability (bedridden) or those with a life-threatening medical condition other than stroke (e.g., active cancer) (Taylor-Piliae, Hoke, et al., 2014).

Study Procedures

For the current study, a determination of human research form was reviewed by the Institutional Review Board (IRB) and concluded that a human subjects review was not required (Appendix B) due to de-identified data for the proposed secondary data analysis. After IRB approval, data were obtained from a secure Box@ UA Health folder and analyzed for relationships outlined in this study’s aims. Of the entire dataset, data were narrowed to include only parameters necessary for the current study: participant descriptive data (e.g. age & gender), MMSE, MoCA, SPI-II scores and patient reported comorbidities (congestive heart failure, diabetes, hypertension, coronary artery disease, dyslipidemia, arrhythmia, major depression and current smoking). Ninety-seven participants were retained from the original 145 due to inclusion of the MoCA scale, which was not collected at the time of the study’s inception but added to the collection methods soon after.

Study Measures

Recurrent Stroke Risk Measure

Several stroke prediction models are available to identify patients most at risk for stroke reoccurrence (e.g., ABCD², RRE-90, SPI-II, & ESRS) (Ay et al., 2010; Chaudhary et al., 2019;
Diener & Frank, 2015). Among them, the Stroke Prognosis Instrument-II (SPI-II) (Appendix E) is a well validated instrument aimed to assess risk of stroke reoccurrence within two years of initial stroke and used in this study (Kernan et al., 2000). The SPI-II instrument was revised (following retesting of SPI-I) using four cohorts, both hospital-based and in three separate randomized controlled trials (WEST, CAPRIE, & UK-TIA). It was validated in retrospective research cohorts, similar to this cross-sectional study’s application. The SPI-II (as in SPI-I) omits the type of stroke as well as presence of aortic plaque burden. It is a practical self-report instrument used in outpatient study settings among study participants with non-disabling ischemic stroke (AUC 0.63) (Kernan et al., 2000).

In this study, participants were categorized according to their SPI-II scores as low, moderate and high. According to Kernan et al. (2000), cutoffs were established observing for stroke outcome rates in the SPI-I study, using log-rank tests across risk groups (Kernan et al., 1991). Additionally, using regression analyses, the authors found that by also including data on congestive heart failure and prior stroke history, the revised instrument further discriminated risk groups compared to the SPI-I (Kernan et al., 2000). It is most relevant to the current study that the SPI-II score increased with each additionally reported comorbidity (diabetes mellitus, severe hypertension, coronary artery disease), and thus may serve as a proxy measure of inflammation due to added comorbidity burden.

**Cognitive Assessment Measures**

Cognitive function was evaluated using two validated instruments: the Mini-Mental State Exam (MMSE) and the Montreal Cognitive Assessment (MoCA) that were reviewed in Chapter 2. These tools are well validated in the literature to measure cognition; yet done so in slightly
different ways to highlight domains of potential brain impairment and with different weightedness. The MoCA has eight domains. Points are deducted for errors. The visuospatial/executive domain (5-points) involves drawing a line in an ordinal or sequenced manner, copying a cube and to drawing a clock. The naming category involves naming three separate animals (lion, rhino & camel) (3-points). Memory involves reading a list of words and recalling those words after five minutes (no-points assigned in this category). Attention involves reading a list of numbers backwards and forward, reading (and tapping simultaneously) a list of letters, and a serial ‘7’ subtraction exercise (6-points). Language involves reading with fluency two sentences and naming the maximum number of words starting with the letter F (3-points). Abstraction describes the similarity between two objects (2-points). Delayed recall is a list of five words, which points are assigned for only uncued, recalled words (5-points). Lastly, orientation points are assigned to date, month, year, day, place and city (6-points). The MMSE instrument is a similar 11 question/activity tool; however, includes following commands such as “please read this and do what it says” (written instructions to close eyes).

**Data Analysis Plan**

All data were assessed for sampling distributions and missing data. Descriptive statistics were computed and tabulated. For Aim 1, the relationship between cognitive function and overall risk for recurrent stroke was determined using Spearman’s correlation test. Also, the relationship between total recurrent stroke risk scores and each cognitive domain assessed by the MoCA and MMSE instruments were determined using Spearman’s correlation test. For Aim 2, differences in cognitive function scores and SPI-II categories were evaluated using Kruskal-Wallis H test. Additionally, differences in cognitive function scores and co-morbid conditions related to the
SPI-II were tested using the Mann-Whitney test. Lastly, for Aim 3, the difference in post-stroke cognitive function were assessed by gender using Mann-Whitney test. In this study, non-parametric tests were used and will be reported in the results because of moderately skewed sample distributions (medians are reported rather than means) and low sample size; however, for completeness, similar results using parametric testing have been included (Appendix A).
CHAPTER 4: RESULTS

The purpose of this secondary data analysis was to examine relationships between cognitive function, and comorbid conditions to ischemic stroke (summarized by SPI-II) using de-identified baseline data collected from 145 stroke survivors.

Characteristics of Participants and Data Set

Some 97 of 145 stroke survivors from the original study were retained in this project (Taylor-Piliae, Hoke et al., 2014). There were no missing data. Age, gender, smoking status and comorbidities are summarized in Table 1.

Table 1

Demographics and Comorbidities of Study Participants (N = 97)

<table>
<thead>
<tr>
<th>Participant Characteristics</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, Mean (SD)</td>
<td>68 (± 9.9)</td>
<td>-</td>
</tr>
<tr>
<td>Participants 70 years or older</td>
<td>46</td>
<td>47.4</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>47.4</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>8</td>
<td>8.2</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>76</td>
<td>78.4</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>66</td>
<td>68.0</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>28</td>
<td>28.9</td>
</tr>
<tr>
<td>Arrhythmia, n (%)</td>
<td>27</td>
<td>27.8</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>23</td>
<td>23.7</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>16</td>
<td>16.5</td>
</tr>
<tr>
<td>Major Depression</td>
<td>15</td>
<td>15.5</td>
</tr>
</tbody>
</table>

Data was first evaluated descriptively by determining dependent variables distributions, shown in Appendix E; MoCA, MMSE, or SPI-II data. Although nonparametric analyses were conducted because of moderate skewness in MoCA and MMSE distributions, parametric testing could have arguably been chosen and results from these tests are included in Appendix F for comparison. Cronbach’s α was then calculated for the instruments used in this population. For the MoCA instrument, the Cronbach’s α was 0.542, lower in this study compared to original
studies (0.83) (Nasreddine et al., 2005). For the MMSE, the Cronbach’s α fell within the range of comparable studies (0.695) indicating a more acceptable level of reliability for this instrument in this sample (Tombaugh, 2005). In this population, the two cognitive instruments, MoCA and MMSE, were significantly correlated (Spearman’s rho correlation coefficient, $r = 0.62, p < 0.0001$), seen in Appendix F, indicating that while both instruments are similar, they are not entirely duplicative as would be indicated if the correlation were very close to 1.0.

**Aim 1 Analysis**

**Aim 1: Evaluate the Relationship between Cognitive Function and Stroke Risk Factors**

**Aim 1a: Examine the associations between participants’ stroke comorbidities (overall SPI-II score) and overall cognitive function (MoCA and MMSE scores).** We show that total SPI-II scores were negatively correlated to both MoCA and MMSE scores ($r = -0.25, p = 0.01$ and $r = -0.22, p = 0.03$, respectively). These data demonstrate that as total SPI-II and the presence of stroke comorbidities increase, cognitive function scores decline. An illustration of these relationships is shown in scatterplots (Appendix E).

**Aim 1b: Examine the relationships between overall stroke comorbidities and the cognitive individual domains captured by the MoCA and MMSE assessments.** Correlations between SPI-II total and the individual cognitive function domains of the MoCA and MMSE were evaluated with findings summarized in Tables 2 and 3 (Appendix F). When corrected for multiple comparisons, the Bonferroni-corrected critical value (alpha) becomes 0.007 (0.05/7 domain comparisons) and 0.005 (0.05/10 domain comparisons), respectively rather than the more typical 0.05. Between both instruments, only one domain from the MoCA instrument demonstrated a statistically significant negative correlation, which was between the score for
recall words and the SPI-II score (Spearman’s rho, r=-0.33, p= 0.001). This negative correlation indicates that as risk of recurrent stroke increased, one’s ability to recall a list of five words without cue declined. Table 3 data analyses for language and reading, are noted with an asterisk, as responses did not vary during testing. For the language score, 100% of participants received two points (all available) for an ability to name a pencil and watch. Likewise, all participants received one point (all available) for the ability to read (and follow directions).

**Table 2**

*Nonparametric Correlations between MoCA Cognitive Function Domains and SPI-II.*

<table>
<thead>
<tr>
<th>MoCA Cognitive Function Domains</th>
<th>SPI-II total risk correlation</th>
<th>Spearman’s rho (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Executive functioning</td>
<td>-0.08 (.421)</td>
<td></td>
</tr>
<tr>
<td>Naming</td>
<td>-0.05 (.620)</td>
<td></td>
</tr>
<tr>
<td>Digit list</td>
<td>-0.03 (.741)</td>
<td></td>
</tr>
<tr>
<td>Repeat sentence</td>
<td>-0.05 (.600)</td>
<td></td>
</tr>
<tr>
<td>Similarity</td>
<td>-0.16 (.106)</td>
<td></td>
</tr>
<tr>
<td><strong>Recall words</strong></td>
<td><strong>-0.33 (.001)</strong></td>
<td></td>
</tr>
<tr>
<td>Orientation</td>
<td>-0.03 (.755)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3**

*Nonparametric Correlations between MMSE Cognitive Function Domains and SPI-II.*

<table>
<thead>
<tr>
<th>MMSE Cognitive Function Domains</th>
<th>SPI-II total risk correlation</th>
<th>Spearman’s rho (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orientation**</td>
<td>0.014 (.891)</td>
<td></td>
</tr>
<tr>
<td>Registration</td>
<td>-0.070 (.497)</td>
<td></td>
</tr>
<tr>
<td>Attention-math**</td>
<td>0.066 (.521)</td>
<td></td>
</tr>
<tr>
<td>Attention-spell**</td>
<td>-0.133 (.194)</td>
<td></td>
</tr>
<tr>
<td>Recall words</td>
<td>-0.210 (.039)</td>
<td></td>
</tr>
<tr>
<td>Language</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Repetition</td>
<td>-0.052 (.612)</td>
<td></td>
</tr>
<tr>
<td>3-stage command</td>
<td>-0.074 (.474)</td>
<td></td>
</tr>
<tr>
<td>Reading</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Writing</td>
<td>-0.100 (.332)</td>
<td></td>
</tr>
<tr>
<td>Copying</td>
<td>-0.062 (.547)</td>
<td></td>
</tr>
</tbody>
</table>

*asterisk indicates no variability during testing.

**The MMSE has seven domains with multiple tasks assigned to one domain for orientation and attention; when able, these domains were analyzed according to their specific task.
Further, in reviewing the data above (Table 2 & 3), the overall negative trend of these individual subdomain correlations demonstrates perhaps some cognitive decline despite not meeting the benchmark for statistical significance.

Aim 2 Analysis

**Aim 2: Examine Differences between Overall Cognitive Function and Individual Co-Morbid Conditions Represented in the SPI-II Assessment Tool**

In Aim 2, we first tested for differences in cognitive function based on the varying presence of comorbidities that may influence post-stroke cognitive recovery and summarized by SPI-II category. In Table 4, average MoCA and MMSE scores are reported for each SPI-II category (low, moderate, & high); these categories represent patient comorbidities, age and gender. For example, participants in the SPI-II high category were patients with comorbidities that have a high prediction for a recurrent stroke, such as congestive heart failure and diabetes mellitus, whereas the participants in the low stroke risk category had comorbid conditions such as hypertension and dyslipidemia (low point predictors). These categories are pre-defined by the SPI-II instrument. We show that there were no significant differences in MMSE scores based on SPI-II categories (Kruskal-Wallis: MMSE: $H=4.069, p = 0.131$). In contrast, there are significant differences in MoCA scores among SPI-II risk groups (Kruskal-Wallis: MoCA: $H = 6.140, p = 0.046$). Specifically, total MoCA scores were smaller (indicative of lower cognitive function) in the moderate risk group when compared to the low risk group (Dunn’s post-hoc test, $p = 0.05$, adjusted; Cohen’s $d=0.43$).
Table 4

Descriptive Table of MoCA and MMSE

<table>
<thead>
<tr>
<th>SPI II</th>
<th>N</th>
<th>MoCA</th>
<th>MMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (SD)</td>
<td>Median (min, max)</td>
</tr>
<tr>
<td>Low (0-3)</td>
<td>48</td>
<td>24.4 (3.98)</td>
<td>25.0 (14, 30)</td>
</tr>
<tr>
<td>Moderate (4-7)</td>
<td>39</td>
<td>22.6 (4.41)</td>
<td>23.0 (11, 30)</td>
</tr>
<tr>
<td>High (8-15)</td>
<td>10</td>
<td>25.0 (2.30)</td>
<td>25.0 (21, 28)</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>23.76 (4.11)</td>
<td>25 (11, 30)</td>
</tr>
</tbody>
</table>

To examine this finding in more detail, a Mann-Whitney U test was conducted for both the MoCA and MMSE instruments to test for differences in cognitive function among patients with and without self-reported histories of congestive heart failure, diabetes mellitus, hypertension, coronary artery disease, dyslipidemia, arrhythmia, major depression or current smoking. Summarized in Table 5, there were no differences in MoCA scores between participants with or without the respective stroke-related comorbid conditions listed. Similarly, as seen in Table 6, there were also no statistically significant differences in MMSE scores between participants with or without stroke-related comorbid conditions. Parametric analyses are included in Appendix F. In summary, there are no cognitive differences in participants, with or without stroke comorbidities when assessed individually.

Table 5

Median MoCA Scores and Stroke Comorbidities: Nonparametric Mann-Whitney U Test.

<table>
<thead>
<tr>
<th>Stroke Comorbidities</th>
<th>Without Condition Median (min, max)</th>
<th>With Condition Median (min, max)</th>
<th>Summary Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>Z</td>
<td>P</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>636.0</td>
<td>-0.12</td>
<td>0.91</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>868.5</td>
<td>-0.78</td>
<td>0.44</td>
</tr>
<tr>
<td>Hypertension</td>
<td>730.5</td>
<td>-0.59</td>
<td>0.55</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>754.5</td>
<td>-0.82</td>
<td>0.41</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>934.0</td>
<td>-0.09</td>
<td>0.93</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>585.0</td>
<td>-0.30</td>
<td>0.76</td>
</tr>
<tr>
<td>Major Depression</td>
<td>336.0</td>
<td>-0.26</td>
<td>0.79</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>25.0 (11, 30)</td>
<td>25.0 (21, 29)</td>
<td>336.0</td>
</tr>
</tbody>
</table>
Table 6

Median MMSE Scores and Stroke Comorbidities: Nonparametric Mann-Whitney U Test

<table>
<thead>
<tr>
<th>Stroke Comorbidities</th>
<th>Without Condition</th>
<th>With Condition</th>
<th>Summary Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (range)</td>
<td>Median (range)</td>
<td>U</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>29.0 (18, 30)</td>
<td>28.5 (25, 30)</td>
<td>609.5</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>29.0 (18, 30)</td>
<td>28.0 (18, 30)</td>
<td>769.0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>29.0 (25, 30)</td>
<td>28.5 (18, 30)</td>
<td>769.0</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>29.0 (18, 30)</td>
<td>28.0 (18-30)</td>
<td>723.5</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>28.0 (24, 30)</td>
<td>29.0 (18, 30)</td>
<td>942.0</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>29.0 (18, 30)</td>
<td>29.0 (24, 30)</td>
<td>909.5</td>
</tr>
<tr>
<td>Major Depression</td>
<td>29.0 (18, 30)</td>
<td>28.0 (25, 30)</td>
<td>606.5</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>29.0 (18, 30)</td>
<td>29.0 (26, 30)</td>
<td>285.0</td>
</tr>
</tbody>
</table>

Aim 3 Analysis

Aim 3: Determine if Post-Stroke Cognitive Function is Different According to Gender in This Population of Community-Dwelling Stroke Survivors

In Aim 3, we examined the effect of gender on post-stroke cognitive function.

Approximately half of the participants were male. Table 7 summarizes the averages for total MoCA and MMSE scores. While females had slightly higher scores on both the MoCA and MMSE compared to males, neither difference was statistically significant ($Z = -1.37, p = 0.17$; $Z = -1.07, p = 0.28$, respectively). These data support that there were no significant gender differences in cognition among community dwelling stroke survivors (Table 7).

Table 7

Average MOCA & MMSE Scores by Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>N</th>
<th>Mean (SD)</th>
<th>Med (min, max)</th>
<th>Mean (SD)</th>
<th>Med (min, max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>46</td>
<td>24.41 (3.60)</td>
<td>25 (15, 30)</td>
<td>28.4 (1.70)</td>
<td>29 (22, 30)</td>
</tr>
<tr>
<td>Males</td>
<td>51</td>
<td>23.17 (4.47)</td>
<td>24 (11, 30)</td>
<td>27.8 (2.51)</td>
<td>28 (18, 30)</td>
</tr>
</tbody>
</table>
CHAPTER 5: DISCUSSION

The overall purpose of this secondary analysis of data collected from community dwelling stroke survivors in a southwest city, was to evaluate the relationships between post-stroke cognitive decline and the presence of stroke comorbid conditions. Secondarily, we evaluated the role of gender in post-stroke cognitive decline in the available data set. We used the SPI-II, a stroke risk assessment scale that tabulated participant stroke comorbidities, as well as the cognitive measurement tools, MoCA and MMSE. The premise for this investigation is the abundance of pre-clinical evidence illustrating chronic inflammation in the brain and a leaky glial scar as mechanisms of post-stroke cognitive decline. Compounding this problem, ischemic stroke does not occur in a vacuum, but rather alongside comorbid conditions that are also common stroke risk factors (e.g., hypertension, diabetes, & coronary artery disease), allowing us to utilize the SPI-II scale. These comorbidities have prominent inflammatory components, increasing systemic inflammation. Taken together, chronic inflammation in the brain as well as from the systemic circulation leaking through an imperfect glial scar, may predispose patients to post-stroke cognitive decline—a relationship we sought to elucidate in the available data set.

In this study, our primary finding was that as total SPI-II score increased (our measure of comorbidity burden) MoCA and MMSE scores decreased (measures of post stroke cognitive decline). When MoCA and MMSE scores were broken down by contributing domain, total SPI-II score was inversely correlated with nearly all domains, with exception to the MoCA recall domain (only domain statistically significant after correction for multiple comparisons). In this data set, participants were classified according to their SPI-II score as low (0-3 points), medium (4-7 points), and high (8-15 points). However, there was no consistent change in either the
MoCA or MMSE scores according to SPI-II classification. In addition, when patients were stratified by presence or absence of singular comorbid conditions in the SPI-II instrument (e.g., those with & without HTN), MoCA and MMSE scores were similar. Lastly, in this data set, MoCA and MMSE scores were not significantly influenced by gender.

We utilized the SPI-II to operationalize the concept of comorbidity burden because the SPI-II measures the presence of inflammatory conditions such as hypertension, coronary artery disease, congestive heart failure and diabetes. Although not prevalent nor well described in the literature, the concept of comorbidity burden is budding as more investigators understand the relationship between disease states and inflammation and the toll that comorbidities have on health, to include cognitive function (Walker, Gottesman, et al., 2019). While comorbidity burden can be physiologically measured (e.g., cytokines, cell types, inflammatory proteins such as CRP) in the setting of various health conditions (heart failure, HTN & diabetes), it is clinically impractical. Therefore, the use of surrogate scales to quantify relationships may help illuminate the inflammatory nature of comorbidity burden and cognitive decline earlier (Walker, Walston, et al., 2019). We considered that the SPI-II score may be a suitable surrogate for comorbidity burden because as this value increased, so too does comorbidity burden. In this data set, we established that there is an inverse relationship between comorbidity burden and cognitive decline. This is not an entirely novel finding in the literature; however, the perspective in the literature is usually quite narrow. For example, most often, studies illustrate differences in cognitive outcomes depending on singular comorbidities, such as diabetes (Palacios-Mendoza et al., 2018) and hypertension (Iadecola & Gottesman, 2019) rather than a collective burden of multiple comorbid conditions. Collectively, the listed co-occurring comorbidities assign a more
deleterious risk of a recurrent stroke and are used to operationalize inflammation in our study as described as a mechanism for cognitive decline in the literature (Adamski et al., 2018; Kwon et al., 2015; Xia et al., 2020). Therefore, this study moves beyond viewing stroke patients as having singular comorbidities, which is less common than those with multiple. The concept of comorbidity burden may be more relevant to the human condition in the context of post-stroke cognitive function or decline than the often-studied narrow perspective.

We next used the SPI-II classifications of low, medium and high to investigate the specific patient attributes (accounted for in the SPI-II) that may account for differences in post-stroke cognitive decline. Our findings did not help to delineate a clear relationship. With a clear relationship, we would have expected that participants in both the moderate and high SPI-II groups would have had lower cognitive values than the low SPI-II group. However, only the participants with moderate SPI-II scores had smaller cognitive scores than the low SPI-II group. It may be that the weighted nature of the SPI-II instrument limits this understanding. Therefore, it bears mentioning on how this instrument scores the comorbidities and additional factors. Using this instrument, participants with congestive heart failure, diabetes, and history of a prior stroke (presence of stroke before original intervention study) were assigned three-points. Two points were assigned to participants over 70 years of age and/or with a history of transient ischemic attack (TIA). One point was then assigned to participants with severe hypertension (systolic blood pressure over 180mmHg) and/or coronary artery disease. A high percentage (78%) of our study’s participants had a history of hypertension (1-point) and nearly 50% were over 70 years of age (2-points). For example, a 71-year-old participant with hypertension may still have significant systemic inflammation that may influence the development of post-stroke cognitive
decline and yet, were classified in the low category. Additionally, history of a prior stroke (N=12) and transient ischemic attack (TIA) (N=44) are included in the SPI-II instrument. Based on this distribution, nearly half of the participants had a TIA (2-point) compared to prior stroke (3-points), assigning them to a lower risk, absent other cumulative comorbidities. These examples highlight the limitation of the SPI-II to properly operationalize the concept of comorbidity burden. Rather, a scale that tabulated and/or weighted comorbidities according to their “level” of inflammation may have better served the purpose of this study. To our knowledge, such a scale has not yet been developed but may be developed based on the pre-clinical and clinical literature relating inflammation and inflammatory conditions to cognition (Walker, Gottesman et al., 2019; Walker, Walston et al., 2019). Walker et al., (2019) used an inflammatory composite score using four blood biomarkers (fibrinogen, white blood cells, von Willebrand factor, factor VIII & CRP) to examine the relationship between the inflammatory nature of diseases and measures of memory, executive function and language. Similar demographics were collected to include additional comorbidities such as cancer and chronic obstructive pulmonary disease (COPD) to our study’s cardiovascular comorbid conditions. These authors found that markers of inflammation were associated with cognitive decline, but their study was significantly limited by their measures of cognitive assessment (only three measures) and large sample attrition.

Our data did show that in this population, participants’ cognitive scores were similar regardless of comorbid conditions evaluated singularly. Therefore, it is possible that there is a type of threshold of comorbidity burden that must be met before post-stroke cognitive decline occurs. On the other hand, it may not be the number of comorbidities or magnitude of
comorbidity burden, but rather the quality or the specific nature of inflammation that is the most important consideration for post-stroke cognitive decline (Filiano, Gadani, & Kipnis, 2017; Metti et al., 2014). To directly compare our findings to the literature, others have shown that those with a singular disease, such as diabetes, have worse cognitive outcomes than those without diabetes (Davis, Zilkens, Starkstein, Davis, & Bruce, 2017). A similar phenomena was observed for hypertension (Iadecola & Gottesman, 2019). Additionally, in the literature, those with a comorbid condition (HTN, diabetes & CHF) were younger at index dementia, particularly in the setting of a longitudinal design (Davis et al., 2017; Kuller et al., 2016). Another aspect of longitudinal designs is the mean time to index dementia, which appears to be approximately 4 years (Zhang et al., 2019). Our study used a cross-sectional design, was not able to capture this change over time. Another primary difference between these studies and this secondary data analysis is the level of physical and cognitive function in our sample; inclusion criteria limited degree of disability due to the study’s intent to examine an exercise intervention.

In this study, participants were community dwelling stroke survivors with good physical and cognitive function. The average MoCA score was approximately 24; and the mean MMSE score of this community dwelling stroke survivors was 28 (on a scale of 1-30). Clinically speaking, a score of 25 or less on either the MoCA or MMSE is indicative of need for a referral to specialist care. This means that by using the MMSE alone, this population would be considered cognitively intact; however, by using the MoCA, this population demonstrates early, mild cognitive impairment and referral for formal evaluation. MoCA is more sensitive than the MMSE in screening for early cognitive impairment post-stroke (Dong et al., 2010). Yet similarly, Dong et al. (2010), found that the MoCA could discriminate subtest scores in the area
of recall. These authors additionally found the MoCA to differentiate subtest scores in the areas of visuospatial/executive functioning and attention compared to the MMSE (Dong et al., 2010; Nys et al., 2005). The poorer performance of recall on the MMSE may be due to its impaired ability to test for complex cognitive impairments in areas of executive functioning, attention and delayed recall. The recall test items on the MMSE (3-item recall) are not as challenging as the items included on the MoCA (5-item recall), which includes two additional tests, the digit span and vigilance/letter tapping (Pendlebury, Cuthbertson, Welch, Mehta, & Rothwell, 2010). This may in part account for the findings in this study, where we observed a relationship between recall and SPI-II when using the MoCA but not the MMSE. A limitation of this study is that stroke location was not recorded in this study. Location and size of stroke lesions can uncover relationships between comorbid conditions and cognitive decline as some regions may be more resilient (or susceptible) to neurodegeneration, perhaps even more so with time (Alexander et al., 2010).

Gender is an important biological factor in stroke science. In our study, we did not observe gender differences in MoCA or MMSE scores among our community dwelling stroke survivors. Gender-related data regarding post-stroke cognitive decline are contradictory. In one study, there was a modest association between gender and cognitive decline; women were more likely to have cognitive decline than men using the MoCA instrument at 6-months post stroke (Mellon et al., 2015). On the other hand and with more recovery, the inverse was seen with the male gender being associated with cognitive decline when measured four years post-stroke (Mahon et al., 2017). Granted geographical locations of these studies varied, for example Ireland compared to New Zealand, respectively. In rural China, cardiovascular and social predictors
were similar between genders; however, men became more susceptible to cognitive decline after the loss of a spouse (Zhang et al., 2019). Singleness, as an independent predictor for cognitive decline among men is seen consistently in other research (Mahon et al., 2017) and may be a confounder to gender differences.

**Study Limitations and Strengths**

In addition to the strengths and limitations discussed in previous paragraphs, the most obvious limitation of this study is that the original study was not designed to address the research questions posited in this secondary data analysis. In this study, the SPI-II instrument was used as a surrogate marker for comorbidity burden and a marker for the degree of systemic inflammation compared to its originally intended use as a stroke prediction system (Kernan et al., 1991). This was not the intended use of the SPI-II despite each predictor’s theoretical association with the pro-inflammatory milieu. Perhaps, as we suggest above, this scale did not adequately operationalize comorbidity burden. Moreover, this study did not directly measure systemic inflammation through well-established biomarkers (e.g., C-reactive protein & cytokines IL-6 & TNFα or hyperdensities noted on MRI or PET imaging). Lastly, the location of brain injury was not noted in this study, which may be important in relation to the MoCA cognitive assessment domains (recall, etc.).

Further limitations of this study include generalizability. The original study was a convenience sample, with participants derived from a number of outpatient centers and offices, who volunteered to participate in the study and met eligibility standards. We can only generalize these data to regions like the desert Southwest or Greater Tucson area, among community-
dwelling stroke survivors. Additionally, the original study was powered for a different set of research questions and therefore the sample size may not be sufficient for this study.

A strength of this study was the inclusion of two cognitive instruments, highlighting the sensitivity of the MoCA in detecting impaired recall (over the MMSE), which was done by investigating correlations within cognitive domains. As stated, the MMSE’s strength is detecting delirium and dementia, part of the excluded criteria in the original study. While the MMSE has been the gold standard in detecting cognitive decline, early cognitive changes may be less identifiable. Several studies have illustrated cognitive decline using cognitive testing (MMSE included) however individual cognitive subtest domains are less often included (Low et al., 2020).

**Conclusions**

Although this secondary data analysis has inherent limitations, these data support the premise for studying comorbidity burden and its effect on cognitive decline. We propose a prospective design with broader sampling, in a more diverse population, compared to participants recruited for a physical intervention study. While the concept of comorbidity burden is emerging (Jiang, Morgenstern, Cigolle, Claflin, & Lisabeth, 2020), the specific tie to inflammation is limited to a few instances (Lin et al., 2019). Because inflammation is a key component of cognitive decline, the link to comorbidity burden to inflammation is key in this context. We suggest that future investigation into operationalizing this concept for an improved measurement and usefulness to understanding cognitive decline in complex patients, such as those recovering from ischemic stroke, would be useful. Application of such a measure may
provide more timely knowledge of the risk of cognitive decline with a threshold of earlier management, or potentially more aggressive management, of co-occurring comorbid conditions.
APPENDIX A:

THE UNIVERSITY OF ARIZONA INSTITUTIONAL REVIEW BOARD APPROVAL LETTER
Date: November 20, 2019
Principal Investigator: Melissa Michaels McElroy
Protocol Number: 1911170564
Protocol Title: Examining the associations between post-stroke cognitive function and recurrent stroke risk factors that include co-morbid conditions.

Determination: Human Subjects Review not Required

Documents Reviewed Concurrently:
- HSPP Forms/Correspondence: Determination of human research-MM HMv2.pdf

Regulatory Determinations/Comments:
- Not Human Subjects Research as defined by 45 CFR 46.102(e): as presented, the activities described above do not meet the definition of research involving human subjects as cited in the regulations issued by the U.S. Department of Health and Human Services which state that "Human subject means a living individual about whom an investigator (whether professional or student) conducting research: (i) Obtains information or biospecimens through intervention or interaction with the individual, and uses, studies, or analyzes the information or biospecimens; or (ii) Obtains, uses, studies, analyzes, or generates identifiable private information or identifiable biospecimens."

The project listed above does not require oversight by the University of Arizona.

If the nature of the project changes, submit a new determination form to the Human Subjects Protection Program (HSPP) for reassessment. Changes include addition of research with children, specimen collection, participant observation, prospective collection of data when the study was previously retrospective in nature, and broadening the scope or nature of the study activity. Please contact the HSPP to consult on whether the proposed changes need further review.

The University of Arizona maintains a Federalwide Assurance with the Office for Human Research Protections (FWA #00004218).
APPENDIX B:

APPROVAL FOR USE OF SECONDARY DATA
October 22, 2019

Re: New IRB application titled “Examining the associations between post-stroke cognitive function and recurrent stroke risk factors that include co-morbid conditions.”

Dear IRB Review Committee,

The proposed study “Examining the associations between post-stroke cognitive function and recurrent stroke risk factors that include co-morbid conditions”, is a secondary data analysis of de-identified baseline data from 145 community-dwelling stroke survivors previously enrolled in my research study: “The Effect of Tai Chi on Physical Functioning and Health-Related Quality of Life in Adult Stroke Survivors (University of Arizona IRB protocol approval #0800000257, PI: Dr. Ruth Taylor-Piliae).

I agree to provide Melissa McElroy, PhD student at the University of Arizona, College of Nursing, an excel file with de-identified data relevant to the completion of her proposed study. Specifically, she will be provided with de-identified baseline data for the following variables: age, gender, cognitive function scores (Montreal Cognitive Assessment), stroke prognosis instrument II scores (SPI-II), along with self-reported hypertension, dyslipidemia, atrial fibrillation, diabetes, major depression, stroke type and sleep disorders.

I am a member of Melissa McElroy’s dissertation committee. Additionally, I ask that her other dissertation committee members (Drs. Helena Morrison, Chair and Janet Rother) be given permission to assist with data management and analysis of all acquired data, specifically for completion of her degree requirements.

Please feel free to contact me, if you have additional questions.

Kind Regards,

Ruth E. Taylor-Piliae, PhD, RN, FAHA, FAAN
Associate Professor and
Robert Wood Johnson Foundation Nurse Faculty Scholar Alumna
College of Nursing, The University of Arizona
1305 N. Martin, P.O. Box 210203
Tucson, AZ 85721-0203
Tel: (520) 626-4881
Fax: (520) 626-4062
Email: rtaylor@nursing.arizona.edu or rtpiliae@email.arizona.edu
APPENDIX C:

FUNCTIONAL STROKE SCALES
**Modified Rankin Score**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No symptoms</td>
</tr>
<tr>
<td>1</td>
<td>No significant disability: able to carry out all usual activities despite some symptoms</td>
</tr>
<tr>
<td>2</td>
<td>Slight disability: able to look after own affairs without assistance but unable to carry out all previous activities</td>
</tr>
<tr>
<td>3</td>
<td>Moderate disability: requires some help but able to walk unassisted</td>
</tr>
<tr>
<td>4</td>
<td>Moderately severe disability: unable to attend to own bodily needs without assistance and unable to walk unassisted</td>
</tr>
<tr>
<td>5</td>
<td>Severe disability: requires constant nursing care and attention, bedridden, incontinent</td>
</tr>
<tr>
<td>6</td>
<td>Dead</td>
</tr>
</tbody>
</table>

(Sacks et al., 2018)
NIH Stroke Scale

**NIH Stro**

**KE SCALE**

Patient Identification: __________________________
Pt. Date of Birth: __/__/____
Hospital: __________________________
Date of Exam: __/__/____

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days [ ] 3 months [ ] Other: __________________________

Time: __:__:_ _ [ ]am [ ]pm

Person Administering Scale: __________________________

Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).

<table>
<thead>
<tr>
<th>Instructions</th>
<th>Scale Definition</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a. Level of Consciousness: The examiner must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.</td>
<td>0 = Alert; easily responsive. 1 = Not alert; but answerable by minor stimulation to obey, answer, or respond. 2 = Not alert; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped). 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic.</td>
<td></td>
</tr>
<tr>
<td>1b. LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasics and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not &quot;help&quot; the patient with verbal or non-verbal cues.</td>
<td>0 = Answers both questions correctly. 1 = Answers one question correctly. 2 = Answers neither question correctly.</td>
<td></td>
</tr>
<tr>
<td>1c. LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paralized hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomiming), and the result scored (i.e., follows none, one or two commands). Patients with ocular trauma, bandages, or other problems not secondary to aphasia are given suitable one-step commands. Only the first attempt is scored.</td>
<td>0 = Performs both tasks correctly. 1 = Performs one task correctly. 2 = Performs neither task correctly.</td>
<td></td>
</tr>
<tr>
<td>2. Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocerebral) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasics patients. Patients with ocular trauma, bandages, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.</td>
<td>0 = Normal. 1 = Partial gaze palsy; gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present. 2 = Forced deviation, or total gaze paresis not overcome by the oculocerebral maneuver.</td>
<td></td>
</tr>
</tbody>
</table>

Rev 10/1/2003
### NIH Stroke Scale

**Interval:**
- [ ] Baseline
- [ ] 2 hours post treatment
- [ ] 24 hours post onset of symptoms ± 20 minutes
- [ ] 3 months
- [ ] Other

**Visual:** Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if there is a clear-cut asymmetry, including quadrantanopia. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.

0 = No visual loss.
1 = Partial hemianopia.
2 = Complete hemianopia.
3 = Bilateral hemianopia (blind including cortical blindness).

**Facial Palsy:** Ask - or use pantomime to encourage - the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma, bandages, ophthalmal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.

0 = Normal symmetrical movements.
1 = Minor paralysis (flattened nasolabial fold, asymmetry on smiling).
2 = Partial paralysis (total or near-total paralysis of lower face).
3 = Complete paralysis of one or both sides (absence of facial movement in the upper and lower face).

**Motor Arm:** The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-affected arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.

0 = No drift, limb holds 90 (or 45) degrees for full 10 seconds.
1 = Drift; limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support.
2 = Some effort against gravity; limb cannot get to or maintain (if used) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity.
3 = No effort against gravity; limb falls.
4 = No movement.
UN = Amputation or joint fusion, explain: ____________

5. Left Arm

5b. Right Arm

**Motor Leg:** The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-affected leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.

0 = No drift; leg holds 30-degree position for full 5 seconds.
1 = Drift; leg falls by the end of the 5-second period but does not hit bed.
2 = Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity.
3 = No effort against gravity; leg falls to bed immediately.
4 = No movement.
UN = Amputation or joint fusion, explain: ____________

6a. Left Leg

6b. Right Leg

---

Rev 10/1/2003
### NIH Stroke Scale

**Interval:**
- [ ] Baseline
- [ ] 2 hours post treatment
- [ ] 24 hours post onset of symptoms ±20 minutes
- [ ] 7-10 days
- [ ] Other

#### 7. Limb Ataxia:
- **0 = Absent.**
- **1 = Present in one limb.**
- **2 = Present in two limbs.**

UN = Amputation or joint fusion, explain:

#### 8. Sensory:
- **0 = Normal; no sensory loss.**
- **1 = Mild-to-moderate sensory loss; patient feels pinprick is less sharp or is dulled on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched.**
- **2 = Severe to total sensory loss; patient is not aware of being touched in the face, arm, and leg.**

#### 9. Best Language:
- **0 = No aphasia; normal.**
- **1 = Mild-to-moderate aphasia; some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient’s response.**
- **2 = Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient’s response.**
- **3 = Mute, global aphasia; no usable speech or auditory comprehension.**

#### 10. Dysarthria:
- **0 = Normal.**
- **1 = Mild-to-moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty.**
- **2 = Severe dysarthria; patient’s speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mutism/anarthric.**

UN = Intubated or other physical barrier, explain:

---

Rev 10/1/2003
11. Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.

<table>
<thead>
<tr>
<th></th>
<th>0 = No abnormality.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities.</td>
</tr>
<tr>
<td>2</td>
<td>Profound hemi-inattention or extinction to more than one modality: does not recognize own hand or orients to only one side of space.</td>
</tr>
</tbody>
</table>
You know how.

Down to earth.

I got home from work.

Near the table in the dining room.

They heard him speak on the radio last night.
MAMA
TIP – TOP
FIFTY – FIFTY
THANKS
HUCKLEBERRY
BASEBALL PLAYER
Barthel Index

### Barthel Index Scoring Form

<table>
<thead>
<tr>
<th>Category</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feeding</strong></td>
<td></td>
</tr>
<tr>
<td>0 = unable</td>
<td></td>
</tr>
<tr>
<td>5 = needs help cutting,</td>
<td></td>
</tr>
<tr>
<td>spreading butter, etc., or</td>
<td></td>
</tr>
<tr>
<td>requires modified diet</td>
<td></td>
</tr>
<tr>
<td>10 = independent</td>
<td></td>
</tr>
<tr>
<td><strong>Bathing</strong></td>
<td></td>
</tr>
<tr>
<td>0 = dependent</td>
<td></td>
</tr>
<tr>
<td>5 = independent (or in shower)</td>
<td></td>
</tr>
<tr>
<td><strong>Grooming</strong></td>
<td></td>
</tr>
<tr>
<td>0 = needs to help with</td>
<td></td>
</tr>
<tr>
<td>personal care</td>
<td></td>
</tr>
<tr>
<td>5 = independent face/hair/</td>
<td></td>
</tr>
<tr>
<td>teeth/shaving (implements</td>
<td></td>
</tr>
<tr>
<td>provided)</td>
<td></td>
</tr>
<tr>
<td><strong>Dressing</strong></td>
<td></td>
</tr>
<tr>
<td>0 = dependent</td>
<td></td>
</tr>
<tr>
<td>5 = needs help but can do</td>
<td></td>
</tr>
<tr>
<td>about half unaided</td>
<td></td>
</tr>
<tr>
<td>10 = independent (including</td>
<td></td>
</tr>
<tr>
<td>buttons, zips, laces, etc.)</td>
<td></td>
</tr>
<tr>
<td><strong>Bowels</strong></td>
<td></td>
</tr>
<tr>
<td>0 = incontinent (or needs to</td>
<td></td>
</tr>
<tr>
<td>be given enemas)</td>
<td></td>
</tr>
<tr>
<td>5 = occasional accident</td>
<td></td>
</tr>
<tr>
<td>10 = continent</td>
<td></td>
</tr>
<tr>
<td><strong>Bladder</strong></td>
<td></td>
</tr>
<tr>
<td>0 = incontinent, or</td>
<td></td>
</tr>
<tr>
<td>catheterized and unable to</td>
<td></td>
</tr>
<tr>
<td>manage alone</td>
<td></td>
</tr>
<tr>
<td>5 = occasional accident</td>
<td></td>
</tr>
<tr>
<td>10 = continent</td>
<td></td>
</tr>
<tr>
<td><strong>Toilet Use</strong></td>
<td></td>
</tr>
<tr>
<td>0 = dependent</td>
<td></td>
</tr>
<tr>
<td>5 = needs some help, but can</td>
<td></td>
</tr>
<tr>
<td>do something alone</td>
<td></td>
</tr>
<tr>
<td>10 = independent (on and off,</td>
<td></td>
</tr>
<tr>
<td>dressing, wiping)</td>
<td></td>
</tr>
<tr>
<td>**Transfers (Bed to Chair and</td>
<td></td>
</tr>
<tr>
<td>Back)**</td>
<td></td>
</tr>
<tr>
<td>0 = unable, no sitting balance</td>
<td></td>
</tr>
<tr>
<td>5 = major help (one or two</td>
<td></td>
</tr>
<tr>
<td>people, physical), can sit</td>
<td></td>
</tr>
<tr>
<td>10 = minor help (verbal or</td>
<td></td>
</tr>
<tr>
<td>physical)</td>
<td></td>
</tr>
<tr>
<td>15 = independent</td>
<td></td>
</tr>
<tr>
<td><strong>Mobility (On Level Surfaces)</strong></td>
<td></td>
</tr>
<tr>
<td>0 = immobile or &lt; 50 yards</td>
<td></td>
</tr>
<tr>
<td>5 = wheelchair independent,</td>
<td></td>
</tr>
<tr>
<td>including corners &gt; 50 yards</td>
<td></td>
</tr>
<tr>
<td>10 = walks with help of one</td>
<td></td>
</tr>
<tr>
<td>person (verbal or physical)</td>
<td></td>
</tr>
<tr>
<td>&gt; 50 yards</td>
<td></td>
</tr>
<tr>
<td>15 = independent (but may use</td>
<td></td>
</tr>
<tr>
<td>any aid; for example, stick)</td>
<td></td>
</tr>
<tr>
<td>&gt; 50 yards</td>
<td></td>
</tr>
<tr>
<td><strong>Stairs</strong></td>
<td></td>
</tr>
<tr>
<td>0 = unable</td>
<td></td>
</tr>
<tr>
<td>5 = needs help (verbal,</td>
<td></td>
</tr>
<tr>
<td>physical, carrying aid)</td>
<td></td>
</tr>
<tr>
<td>10 = independent</td>
<td></td>
</tr>
</tbody>
</table>

**Total Score =**

(Dewing, 1992)
APPENDIX D:

COGNITIVE ASSESSMENT TOOLS
Montreal Cognitive Assessment

**VISUOSPATIAL / EXECUTIVE**

- **Copy cube**: [ ]
- **Draw CLOCK (Ten past eleven)**: (3 points)

**MEMORY**

- Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

**ATTENTION**

- Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order: [ ] 2 1 8 5 4
- Subject has to repeat them in the backward order: [ ] 7 4 2

**NAMING**

- Contour: [ ]
- Numbers: [ ]
- Hands: [ ]

**LANGUAGE**

- Fluency / Name maximum number of words in one minute that begin with the letter F: [ ] ______ (N ≥ 11 words)

**ABSTRACTION**

- Similarity between e.g. banana - orange = fruit: [ ] train - bicycle: [ ] watch - ruler: [ ]

**DELAYED RECALL**

- Hit to recall words with no cue:
  - Category cue: [ ]
  - Multiple choice cue: [ ]

**ORIENTATION**

- Date: [ ]
- Month: [ ]
- Year: [ ]
- Day: [ ]
- Place: [ ]
- City: [ ]

**TOTAL**: [ ]
# Mini-Mental State Examination (MMSE)

**Patient’s Name:** ____________________________  **Date:** ___________

*Instructions: Score one point for each correct response within each question or activity.*

<table>
<thead>
<tr>
<th>Maximum Score</th>
<th>Patient’s Score</th>
<th>Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>“What is the year? Season? Date? Day? Month?”</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>“Where are we now? State? County? Town/city? Hospital? Floor?”</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>The examiner names three unrelated objects clearly and slowly, then the instructor asks the patient to name all three of them. The patient’s response is used for scoring. The examiner repeats them until patient learns all of them, if possible.</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>“I would like you to count backward from 100 by sevens.” (93, 86, 79, 72, 65, …) Alternative: “Spell WORLD backwards.” (D-L-R-O-W)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>“Earlier I told you the names of three things. Can you tell me what those were?”</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>“Repeat the phrase: ‘No ifs, ands, or buts.’”</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>“Take the paper in your right hand, fold it in half, and put it on the floor.” (The examiner gives the patient a piece of blank paper.)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>“Please read this and do what it says.” (Written instruction is “Close your eyes.”)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>“Make up and write a sentence about anything.” (This sentence must contain a noun and a verb.)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>“Please copy this picture.” (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.)</td>
</tr>
</tbody>
</table>

**TOTAL** 30
**Interpretation of the MMSE:**

<table>
<thead>
<tr>
<th>Method</th>
<th>Score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Cutoff</td>
<td>&lt;24</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Range</td>
<td>&lt;21</td>
<td>Increased odds of dementia</td>
</tr>
<tr>
<td></td>
<td>&gt;25</td>
<td>Decreased odds of dementia</td>
</tr>
<tr>
<td>Education</td>
<td>21</td>
<td>Abnormal for 8th grade education</td>
</tr>
<tr>
<td></td>
<td>&lt;23</td>
<td>Abnormal for high school education</td>
</tr>
<tr>
<td></td>
<td>&lt;24</td>
<td>Abnormal for college education</td>
</tr>
<tr>
<td>Severity</td>
<td>24-30</td>
<td>No cognitive impairment</td>
</tr>
<tr>
<td></td>
<td>18-23</td>
<td>Mild cognitive impairment</td>
</tr>
<tr>
<td></td>
<td>0-17</td>
<td>Severe cognitive impairment</td>
</tr>
</tbody>
</table>

**Interpretation of MMSE Scores:**

<table>
<thead>
<tr>
<th>Score</th>
<th>Degree of Impairment</th>
<th>Formal Psychometric Assessment</th>
<th>Day-to-Day Functioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-30</td>
<td>Questionably significant</td>
<td>If clinical signs of cognitive impairment are present, formal assessment of cognition may be valuable.</td>
<td>May have clinically significant but mild deficits. Likely to affect only most demanding activities of daily living.</td>
</tr>
<tr>
<td>20-25</td>
<td>Mild</td>
<td>Formal assessment may be helpful to better determine pattern and extent of deficits.</td>
<td>Significant effect. May require some supervision, support and assistance.</td>
</tr>
<tr>
<td>10-20</td>
<td>Moderate</td>
<td>Formal assessment may be helpful if there are specific clinical indications.</td>
<td>Clear impairment. May require 24-hour supervision.</td>
</tr>
<tr>
<td>0-10</td>
<td>Severe</td>
<td>Patient not likely to be testable.</td>
<td>Marked impairment. Likely to require 24-hour supervision and assistance with ADL.</td>
</tr>
</tbody>
</table>

**Source:**
APPENDIX E:

RECURRENT STROKE RISK PROFILE
Tai Chi Exercise for Stroke Survivors Study
Recurrent Stroke Risk Profile

ID: ________
Acrostic: ________
Date: ________

Stroke Prognosis Instrument II (SPI-II)¹

The SPI-II is a validated tool for determining risk of recurrent stroke or death within 2 years. A recurrent stroke risk score is calculated based on several variables and assigned points determined by its predictive importance (range= 0-15).

Data will be abstracted from subject’s questionnaire, and physical functioning measures.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Points</th>
<th>Subject’s Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestive Heart Failure</td>
<td>3 points</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>3 points</td>
<td></td>
</tr>
<tr>
<td>Prior stroke</td>
<td>3 points</td>
<td></td>
</tr>
<tr>
<td>Age &gt; 70 years</td>
<td>2 points</td>
<td></td>
</tr>
<tr>
<td>Index Event Stroke versus TIA</td>
<td>2 points</td>
<td></td>
</tr>
<tr>
<td>Severe Hypertension (SBP&gt;180mmHg or DBP&gt;100mmHg)</td>
<td>1 point</td>
<td></td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>1 point</td>
<td></td>
</tr>
</tbody>
</table>

Total Number of Points

Recurrent Stroke Risk (circle one)

Low risk 0-3 points
Medium risk 4-7 points
High risk 8-15 points

APPENDIX F:

SUPPLEMENTARY DATA FIGURES
Supplemental Figure 1. MoCA score frequency distribution.

Supplemental Figure 2. MMSE score frequency distribution.
Supplemental Figure 3. Total Stroke Risk Profile score frequency distribution.

Supplemental Figure 4. Correlation between MoCA and MMSE.
Supplemental Figure 5. Correlation between MMSE and SPI-II.

Supplemental Figure 6. Scatterplot: MoCA and MMSE Fit Line.
APPENDIX G:

SUPPLEMENTARY PARAMETRIC ANALYSES
**Supplemental Table 1. Parametric Correlations between MoCA Cognitive Function Domains and SPI-II.**

<table>
<thead>
<tr>
<th>MoCA Cognitive Function Domains</th>
<th>SPI-II total risk correlation</th>
<th>Pearson’s r value (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Executive functioning</td>
<td></td>
<td>-.06 (.555)</td>
</tr>
<tr>
<td>Naming</td>
<td></td>
<td>.01 (.942)</td>
</tr>
<tr>
<td>Digit list</td>
<td></td>
<td>-.02 (.994)</td>
</tr>
<tr>
<td>Repeat sentence</td>
<td></td>
<td>-.03 (.745)</td>
</tr>
<tr>
<td>Similarity</td>
<td></td>
<td>-.14 (.185)</td>
</tr>
<tr>
<td><strong>Recall words</strong></td>
<td></td>
<td><strong>-.31 (.002)</strong></td>
</tr>
<tr>
<td>Orientation</td>
<td></td>
<td>-.003 (.976)</td>
</tr>
</tbody>
</table>

**Supplemental Table 2. Parametric Correlations between MMSE Cognitive Function Domains and SPI-II.**

<table>
<thead>
<tr>
<th>MMSE Cognitive Function Domains</th>
<th>SPI-II total risk correlation</th>
<th>Pearson’s r value (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orientation</td>
<td></td>
<td>.013 (.897)</td>
</tr>
<tr>
<td>Registration</td>
<td></td>
<td>-.001 (.992)</td>
</tr>
<tr>
<td>Attention-math</td>
<td></td>
<td>.071 (.487)</td>
</tr>
<tr>
<td>Attention-spell</td>
<td></td>
<td>-.092 (.369)</td>
</tr>
<tr>
<td><strong>Recall words</strong></td>
<td></td>
<td><strong>-.188 (.066)</strong></td>
</tr>
<tr>
<td>Language</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Repetition</td>
<td></td>
<td>-.060 (.561)</td>
</tr>
<tr>
<td>3-stage command</td>
<td></td>
<td>-.070 (.497)</td>
</tr>
<tr>
<td>Reading</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Writing</td>
<td></td>
<td>-.076 (.462)</td>
</tr>
<tr>
<td>Copying</td>
<td></td>
<td>-.056 (.584)</td>
</tr>
</tbody>
</table>

*asterisk indicates no variability during testing.
Supplemental Table 3. Differences in MoCA Scores and Stroke Comorbidities: Parametric Student’s T-Test.

<table>
<thead>
<tr>
<th>Stroke Comorbidities</th>
<th>Without Condition</th>
<th>With Condition</th>
<th>Summary Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>t</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>23.75 (4.18)</td>
<td>23.81 (3.86)</td>
<td>0.053</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>23.95 (4.04)</td>
<td>23.28 (4.31)</td>
<td>0.726</td>
</tr>
<tr>
<td>Hypertension</td>
<td>24.47 (3.65)</td>
<td>23.56 (4.23)</td>
<td>0.897</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>23.95 (3.95)</td>
<td>23.17 (4.62)</td>
<td>0.785</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>24.16 (4.08)</td>
<td>23.57 (4.14)</td>
<td>0.652</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>23.68 (4.22)</td>
<td>23.96 (3.87)</td>
<td>-0.296</td>
</tr>
<tr>
<td>Major Depression</td>
<td>23.78 (4.18)</td>
<td>23.66 (3.84)</td>
<td>0.098</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>23.67 (4.22)</td>
<td>24.75 (2.49)</td>
<td>-0.707</td>
</tr>
</tbody>
</table>

Supplemental Table 4. Differences in Mean MMSE Scores and Stroke Comorbidities: Parametric Student’s t test.

<table>
<thead>
<tr>
<th>Stroke Comorbidities</th>
<th>Without Condition</th>
<th>With Condition</th>
<th>Summary Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>t</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>28.02 (2.27)</td>
<td>28.37 (1.62)</td>
<td>-0.587</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>28.32 (1.92)</td>
<td>27.50 (2.64)</td>
<td>0.151</td>
</tr>
<tr>
<td>Hypertension</td>
<td>28.62 (1.16)</td>
<td>27.93 (2.36)</td>
<td>1.283</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>28.21 (2.03)</td>
<td>27.65 (2.57)</td>
<td>1.088</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>28.20 (1.44)</td>
<td>28.03 (2.45)</td>
<td>0.343</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>28.06 (2.35)</td>
<td>28.15 (1.66)</td>
<td>-0.184</td>
</tr>
<tr>
<td>Major Depression</td>
<td>28.05 (2.28)</td>
<td>28.27 (1.53)</td>
<td>0.355</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>28.02 (2.23)</td>
<td>28.75 (1.39)</td>
<td>-0.906</td>
</tr>
</tbody>
</table>
REFERENCES


Dong, Y., Slavin, M. J., Chan, B. P., Venketasubramanian, N., Sharma, V. K., Collinson, S. L., ... Chen, C. L. (2014). Improving screening for vascular cognitive impairment at three to six months after mild ischemic stroke and transient ischemic attack. *Int Psychogeriatr, 26*(5), 787-793. doi:10.1017/S1041610213002457


Liu, Y., Ho, R. C., & Mak, A. (2012). Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: A meta-analysis and meta-regression. *J Affect Disord, 139*(3), 230-239. doi:10.1016/j.jad.2011.08.003


