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Neuroimaging Biomarkers of Alzheimer's Disease and Cerebrovascular Disease in Patients with Subjective Cognitive Concerns and Mild Cognitive Impairment

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Neuroimaging Biomarkers of Alzheimer's Disease and Cerebrovascular Disease in Patients with Subjective Cognitive Concerns and Mild Cognitive Impairment

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Abstract

In these studies, patients with subjective cognitive concerns (SCC; n = 43) and mild cognitive impairment (MCI; n = 18), were compared using neuroimaging biomarkers for β-amyloid, white matter hyperintensities (WMH) of presumed vascular origin, and cerebral perfusion. Higher WMH burden was found in SCC compared to MCI ($p = 0.02$). In the whole cohort, there was a correlation between higher β-amyloid accumulation and lower left ($p = 0.02$) and right temporal gyrus perfusion ($p = 0.05$), while higher WMH burden was associated with lower perfusion in the cortical grey matter ($p = 0.01$), posterior cingulate cortex ($p = 0.02$) and right temporal gyrus ($p = 0.01$). Exploratory comparisons suggested lower perfusion in the left temporal gyrus and anterior cingulate cortex in amnestic MCI (n = 12) compared to non-amnestic MCI (n = 6). These findings suggest that pathologies consistent with dementia are evident in early cognitive decline.

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Table of Contents

List of Tables

List of Figures and Illustrations

List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
ACC	Anterior Cingulate Cortex
AD	Alzheimer's Disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
ADRA	Alzheimer's Disease and Related Disorders Association
AHA	American Heart Association
aMCI	Amnestic MCI
APOE	Apolipoprotein
ASL	Arterial Spin Labeling
CBF	Cerebral Blood Flow
CDR	Clinical Dementia Rating
CMB	Cerebral Microbleeds
CSF	Cerebral Spinal Fluid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders-IV
DVR	Distribution Volume Ratio
FDG	Fluorodeoxyglucose
FSL	FMRIB Software Library
GM	Grey Matter
IQR	Interquartile Range
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Examination
MNI	Montreal Neurological Institute
MR	Magnetic Resonance
naMCI	Non-amnestic MCI
NFT	Neurofibrillary Tangles
NIA-AA	National Institute on Aging-Alzheimer's Association
NINCDS	National Institute of Neurological and Communicative Disorders and Stroke
OFC	Orbitofrontal Cortex
PCC	Positron Emission Tomography
PET	Posterior Cingulate Cortex
PiB	Pittsburgh Compound B
PVE	Partial Volume Effect
RF	Radiofrequency
ROI	Region of Interest
SCC	Subjective Cognitive Concerns
SCD	Subjective Cognitive Decline
SCI	Subjective Cognitive Impairment
SD	Standard Deviation
SMI	Subjective Memory Impairment
	Suspected Non-amyloid Pathology

SNAP	Single-Photon Emission Computed Tomography
SPECT	Subcortical Vascular Dementia
STRIVE	STandards for Reporting Vascular Changes in nEuroimaging
SVaD	Small Vessel Disease
SVD	T2-Weighted FLuid Attenuated Inversion Recovery
T2-FLAIR	Vascular Dementia
VaD	Vascular Cognitive Impairment
VCI	World Health Organization
WHO	White Matter Hyperintensity
WMH	

Chapter One: **INTRODUCTION**

1.1 Introduction

The World Health Organization (WHO) estimates a two-fold increase in the number of people living with dementia within the next 15 years, which translates to approximately 66 million people affected worldwide. The two most common causes of dementia, Alzheimer's disease (AD) and vascular dementia (VaD) account for 70% and 20% of all cases, respectively. While the number of deaths related to cancer and cardiovascular diseases have declined in the past decade, the number of deaths related to Alzheimer's disease have been increasing (Thies *et al*., 2013). Despite existing treatments for symptoms of dementia, dementia is not curable. Therefore, better understanding of potential causes and treatment of dementia will help us to more readily respond to this daunting projection proposed by WHO.

The focus of this thesis is to explore in greater depth the prodromal stages of dementia, so there can be better tools to identify and prevent early cognitive decline. In this introductory chapter, I introduce concepts and terms used to describe cognitive symptoms and impairments, and describe briefly the two most common causes of pathological cognitive impairment, AD and vascular cognitive impairment (VCI). Chapter 2 provides a general outline of the study methodology. In subsequent chapters, I provide more specific background information to justify my analyses and conclusions (Chapters 3 to 5).

1.2 Cognitive Syndromes and Causes

Dementia is defined as the presence of cognitive decline in one or more domains (*i.e.,* memory, language) past the point where it interferes with daily functioning. Within the past couple of decades, an at-risk stage of dementia has been defined, termed mild cognitive impairment (MCI).

Albert *et al.* (2011) defines MCI has having subjective concerns over memory or cognition with objective evidence of cognitive impairment in one or more domains, but essentially preserved activities of living. Preservation of daily function is what discriminates MCI from dementia. MCI is a heterogeneous entity that can be further classified into four clinical subtypes depending on the cognitive function and number of domains impaired: amnestic single-domain, amnestic multiple-domain, non-amnestic single domain and non-amnestic multiple domain (Figure 1-1). Specifically, amnestic MCI is characterized by memory impairment, compared to non-amnestic MCI, which is characterized by impairment in other cognitive domains, such as processing speed, executive function and language (Winblad *et al*., 2004). Longitudinal studies examining the progression of MCI subtypes suggests that both the amnestic and multiple-domain MCI have the highest likelihood of converting to AD (Forlenza *et al*., 2009; Jungwirth *et al*., 2012; Lee *et al*., 2012). According to memory clinic-based studies, the progression of MCI to dementia occurs at a rate of 10 to 15% annually (DeCarli 2003; Panza *et al*., 2005).

In addition to the growing literature on MCI, others have ventured into studying a population of individuals who report cognitive concerns despite having normal performance on standardized neuropsychological testing. Since they score within cognitively healthy range on neuropsychological testing, these individuals do not have MCI. Donovan *et al*. (2014) used the term "subjective cognitive concerns" (SCC) to describe this group, which is analogous to other terms that have been used in literature, including subjective cognitive decline or impairment. Recent evidence suggests that there is greater risk for patients with SCC, compared to healthy counterparts, to experience future cognitive decline (Jessen *et al*., 2014).

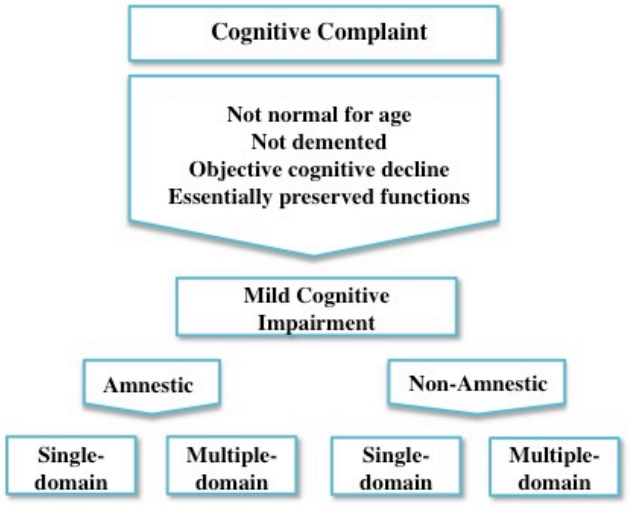

Figure 1-1 Classification of mild cognitive impairment based on the original Petersen *et al.* (1999) criteria, which was revised by Winblad *et al.* (2004) to include amnestic and non-amnestic subtypes of MCI.

Dementia and MCI can have multiple causes, but the two most common causes, accounting for the majority of cases, are AD and VaD. Although VCI and VaD have been used interchangeably in literature, we refer to American Heart Association (AHA) criteria when classifying vascular dementia as one of the causes of VCI (Gorelick *et al*., 2011).

Neuropathologically, AD is marked by the accumulation of β-amyloid plaques, neurofibrillary tangles and downstream cell death and atrophy. The definition of AD dementia proposed by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) was most recently revised in 2011. According to the NINCDS-ADRDA criteria, AD dementia can be categorized into *probable* AD dementia, *possible* AD dementia and *probable* or *possible* AD dementia with evidence of AD pathophysiology. The core criteria of all these categories involve cognitive impairment of one or more domain, resulting in functional decline (independent of

psychiatric disorders) that impairs activities of daily living. *Probable* AD dementia represents the most prototypic diagnosis of AD. It is described as an insidious onset of amnestic and/or non-amnestic cognitive decline, and free of substantial concomitant cerebrovascular disease, vascular pathology or Lewy bodies.

VaD is the second most common cause of dementia. Neuropathologically, VaD is marked by vascular injury to the brain including strokes and white matter lesions of presumed vascular origin. Under the American Heart Association (AHA) criteria, all forms of cognitive deficits with a vascular origin are classified under the term VCI, which include VaD as well as vascular mild cognitive impairment (Gorelick *et al*., 2011). In *probable* VaD, there is a clear link between the cognitive impairment and onset of a vascular event (*i.e*., stroke) or a pattern and severity of subcortical cerebrovascular disease can be clearly identified. Despite overlapping clinical diagnostic criteria for AD and VaD, these two leading causes of dementia have unique etiologies (Sahathevan *et al*., 2012). As alluded to in the NINCDS-ADRDA diagnostic criteria, AD and VaD can also co-exist as mixed dementia, and therefore making it difficult to pinpoint the origin of cognitive decline in these cases (Chui *et al*., 2006).

In contrast to dementia and MCI, much less is known about the causes of SCC. It is speculated that SCC may be influenced by personality traits and mood disorders in addition to underlying pathologies related to dementia. In this thesis, we have undertaken experiments designed to better elucidate differences in the pathological causes of SCC and MCI.

1.3 Clinical Characteristics of Mild Cognitive Impairment

1.3.1 Cognitive Profile of Mild Cognitive Impairment

As outlined by the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria, MCI is characterized by objective evidence of impaired performance on neuropsychological assessments, but without impact on performance of activities of daily living (Albert *et al*., 2011). A cut off of -1.0 or -1.5 standard deviations on neuropsychological testing is often used to satisfy the criterion of objective evidence of impairment (Petersen *et al*., 1999). The heterogeneous nature of MCI allows for stratification into clinical subtypes based on neuropsychological test performance. Amnestic MCI refers to impairment of the memory domain, whereas non-amnestic MCI refers to impairment of non-memory domains including attention, visuospatial ability, processing speed, language and executive function. Depending on the number of domains affected, MCI can be further classified into single- and multiple-domains.

1.3.2 Etiology of Mild Cognitive Impairment

MCI represent a prodromal state of dementia, which is a syndrome with various causes. Therefore, pathophysiological processes underlying MCI include those of AD, cerebrovascular disease, frontotemporal dementia, dementia with Lewy bodies, Parkinson's disease, Huntington's disease, HIV/AIDS, traumatic brain injury and substance abuse (Petersen *et al*., 2014).

The most common and well-described etiology of MCI is AD, where β-amyloid plaques accumulate in the neocortical regions. As the disease progress to a denser collection, diffuse plaques become senile neuritic plaques (Markesbery *et al*., 2006). In addition to β-amyloid plaques, increased neurofibrillary tangles (NFTs) are observed in the amygdala, entorhinal

cortex, subiculum and inferior parietal cortex (Guillozet *et al*., 2003, Nelson *et al*., 2009). In contrast, vascular MCI results from a symptomatic stroke or subcortical ischemic pathology, including white matter lesions and silent brain infarcts (Gorelick *et al*., 2011).

1.4 Current Understanding of Subjective Cognitive Concerns

Although our understanding is still in its infancy, a growing number of studies attempt to describe the prognosis of patients who self-report cognitive decline despite normal neuropsychological test performance. These studies suggest that patients with SCC are at a greater risk of future cognitive decline (Glodzik-Sobanska *et al*., 2007) and that the decline occurs at an accelerated rate when compared to the healthy aging population (Reisberg *et al*., 2010). Some studies have speculated that standardized neuropsychological assessments designed to diagnose MCI are insensitive for detecting early changes found in SCC, but that the deficits indeed exist compared to cognitively healthy adults (Reisberg and Gauthier, 2008).

The etiology of SCC has not been described extensively in literature compared to MCI. Both AD pathologies (Perrotin *et al*., 2012) and vascular risk factors underlying dementia (Paradise *et al*., 2011) have been cited as likely contributors to SCC etiology. However, others propose the influence of factors such as depression, anxiety and personality traits on the tendency to self-report cognitive decline (Donovan *et al*., 2014). Multiple studies accounting for the possible influence of these factors still reported higher risk of cognitive decline in SCC compared to the healthy aging population (Snitz et al., 2008 2011, Amariglio et al., 2011).

Limited neuroimaging studies suggest that there may be both volumetric and metabolic changes in the SCC population. Mosconi *et al*. (2008) found both hippocampal and entorhinal atrophy and hypometabolism in the parahippocampal gyrus in patients with self-perceived

memory concerns. Additional studies also suggest decreased grey matter (GM) volume (Saykin *et al*., 2006, van Norden *et al*., 2008) and AD-specific pattern of GM atrophy in patients with SCC (Peters *et al*., 2014). Aim 1 of this thesis will provide a more in-depth discussion of the neuroimaging studies in SCC.

1.4.1 Relationship of SCC to Preclinical AD

Since pathological processes leading to AD begin decades prior to the diagnosis, many studies attempt to identify biomarker evidence and subtle cognitive changes during the preclinical AD stage. Preclinical AD is defined as the stage where evidence of AD pathological process is present, but the patient does not have MCI or dementia. The Preclinical Working Group of NIA-AA recently proposed a conceptual framework that can be used as operational research criteria for AD. The criteria stratify preclinical AD into three stages (Sperling *et al*., 2011). Stage one is characterized with biomarker evidence of β-amyloid, and during stage two, evidence of neurodegenerative changes (*i.e*., brain atrophy, hypometabolism or elevated CSF tau) begin to accrue. In stage three, cognitive and behavioural symptoms become evident in the presence of β-amyloid and neurodegenerative changes.

Though there is limited data on changes found in preclinical AD, Pittsburgh Compound B (PiB)-PET has shown to be a sensitive *in vivo* method for detecting β-amyloid changes. Cognitively healthy adults who are PiB-positive perform slightly worse on episodic memory, and have an accelerated rate of cortical atrophy compared PiB-negative individuals (Chetelat *et al*., 2012). CSF evidence, including β-amyloid, total tau and hyperphosphorylated tau levels, were also good predictors of memory and executive function decline in preclinical AD (van Harten *et al*., 2013). These studies showing that AD biomarkers are associated with cognitive changes

even in patients without MCI or dementia, making it plausible that preclinical AD would be one of the potential causes of SCC.

1.5 Neuroimaging Biomarkers of Cognitive Impairment and Dementia

There have been many neurochemical, neuroimaging and genetic biomarkers of neuropathology identified that serve as good indicators of patients at greater risk of cognitive decline. Neuropsychological tests can identify cognitive decline (Cullen *et al*., 2007), but neuroimaging biomarkers provide additional information on the cause and prognosis. Furthermore, neuropsychological test results may lie within population normal ranges despite the presence of cognitive symptoms, as in SCC. In patients with SCC, neuroimaging may identify radiological changes, which correlate to preclinical pathological changes yet to manifest as lowered neuropsychological test performance. Using neuropsychological testing alone to predict conversion to AD has been criticized for being circular, since the diagnosis was also based on the same psychometric tests.

Neuroimaging biomarkers provide *in vivo*, and often non-invasive radiologic assessment of neuropathological changes that lead to cognitive decline. Although neuroimaging markers suggest underlying neuropathologies, autopsy remains the gold standard for confirming diagnosis of AD or cerebrovascular disease.

The aims of this thesis are focused on exploring three different neuroimaging biomarkers of dementia in SCC and MCI, and MCI subtypes, while investigating the relationship between these biomarkers in the context of early stages of cognitive decline. To account for the two most common etiology underlying dementia, we quantified β-amyloid accumulation with Pittsburgh Compound B PET (PiB-PET) and white matter lesions manifesting as white matter

hyperintensity (WMH) with T2-weighted fluid-attenuated inversion recovery (FLAIR) MR. We also measured the correspondent cortical and regional cerebral perfusion using arterial spin labeling (ASL) MR.

1.5.1 Imaging Cerebral β-amyloid Accumulation with PiB-PET

Ten years ago, Klunk *et al*. (2004) became the first group to use the ^{11}C- PiB-PET imaging to detect AD pathology *in vivo*. Prior to this neuroimaging technique, β-amyloid plaques were quantified using post-mortem analyses. PiB, the exogenous tracer, has a great binding affinity for β-amyloid, and has been shown to highlight β-amyloid deposition in the association cortex of patients suspected of AD (Klunk *et al*., 2004). Similarly, PiB retention patterns in MCI, especially of the amnestic subtype, resemble that of AD (Price *et al*., 2005; Pike *et al*., 2007). Longitudinal studies suggest that higher PiB retention in MCI is also correlated to a greater likelihood of conversion to AD (Forsberg *et al*., 2008; Jack *et al*., 2010; Villemagne *et al*., 2011). Furthermore, the utility of PiB-PET have also been demonstrated in the preclinical asymptomatic stage, where cerebral β-amyloid accumulation can be present as early as 15 years prior to the diagnosis of AD (Rowe *et al*., 2010; Perrotin *et al*., 2012; Villemagne *et al*., 2013). However, the specificity of PiB binding has been criticized since some reports binding to both diffuse β-amyloid plaques and cerebrovascular amyloid angiopathy in additional to senile plaques (Lockhard *et al*., 2007).

1.5.2 Imaging White Matter Lesions using T2-FLAIR MR

On T2-FLAIR images, white matter lesions manifest as areas of hyperintense signals called white matter hyperintensity (WMH). White matter lesions, a consequence of small vessel

disease, are thought to occur as a result of deep cerebral vessel lumen restriction that leads to hypoperfusion of white matter and demyelination (Pantoni 2010). WMH have been identified in cognitively healthy adults (Schneider *et al*., 2003), but higher WMH burden predicts faster rate of cognitive decline (De Groot *et al*., 2002; Prins *et al*., 2005) and subsequent conversion to dementia (Vermeer *et al*., 2003; Prins *et al*., 2004). In patients with AD pathology, co-existing white matter lesions are often associated with lower cognitive test performance (Heyman *et al*., 1998), although this finding has been inconsistent (Lee *et al*., 2000; Jellinger 2001). Studies examining the longitudinal relationship between WMH burden and cognitive performance reports greater decline in both executive function and processing speed (de Groot *et al*., 2001; Prins *et al*., 2005; Vannorsdall *et al*., 2009).

1.5.3 Imaging Cerebral Perfusion using Arterial Spin Labeling MR

Arterial spin labeling (ASL) MR is a novel and direct method of assessing cerebral perfusion compared to surrogate markers such as fluorodeoxyglucose-positron emission tomography (FDG-PET) and blood oxygen level dependent functional MRI (BOLD fMRI). ASL is a non-invasive MR technique that measures cerebral perfusion using magnetically labeled blood as a freely diffusible tracer (Detre *et al*., 2012). Although the exact cause-effect relationship remains unclear, reduced cerebral blood flow often accompanies underlying pathology in MCI, AD and VCI.

Patterns of hypoperfusion in MCI and AD occur in a region dependent manner. Johnson *et al*. (2005) reports decreased perfusion in the bilateral posterior cingulate gyri, bilateral middle frontal gyri and parietal association cortices of AD patients, and right parietal lobe in MCI patients. Using voxel-based analyses, Dai *et al*. (2009) showed decreased perfusion in the

posterior cingulate cortex and medial precuneus in both MCI and AD compared to healthy controls. Ding *et al*. (2014) recently showed similar cross-sectional findings where AD patients exhibited decreased perfusion in the bilateral temporo-parieto-occipital cortices and left limbic lobe, and amnestic MCI patients exhibited decreased perfusion in the left occipital lobe, bilateral inferior temporal cortex and right middle temporal cortex. Very few studies have delineated MCI based on subtypes when studying patterns of cerebral hypoperfusion. Within *a priori* ROIs, hypoperfusion in the middle frontal cortex, left precuneus and bilateral posterior cingulate was found in MCI patients with executive dysfunction compared to healthy controls (Chao *et al*., 2009).

Interestingly, increased cerebral perfusion has also been implicated in MCI and early AD, and is thought to reflect a compensatory mechanism during earlier stages of cognitive decline. Compared to age-matched healthy controls, patterns of hyperperfusion have been detected in the anterior cingulate cortex and hippocampus of AD, and the hippocampus of MCI (Alsop *et al*., 2008, Dai *et al*., 2009). Hyperperfusion has also been found in the bilateral frontal lobes and right temporal subgyral region of amnestic MCI compared to healthy controls (Ding *et al*., 2014).

1.6 Study Aims and Hypothesis

The focus of my thesis is to characterize SCC and MCI using three neuroimaging biomarkers, (β-amyloid by PiB-PET, WMH by T2-FLAIR imaging and perfusion by ASL imaging described in Section 1.5), that highlight important etiological and pathological changes found in AD and cerebrovascular disease. By simultaneously comparing β-amyloid retention with PiB-PET, white

matter lesions with T2-FLAIR and cerebral perfusion with ASL MR imaging, we hope to elucidate key differences between SCC and MCI, and between MCI subtypes.

Study Aim 1 We will compare PiB retention, WMH burden and ASL perfusion in patients with subjective cognitive concerns and mild cognitive impairment to characterize differences in β-amyloid deposition, white matter lesions and cortical and regional cerebral perfusion, respectively.

Hypothesis 1 MCI represents a later stage of cognitive decline than SCC, because MCI patients have objective evidence of impairment on neuropsychological testing. We hypothesize that patients with MCI will exhibit greater PiB retention, higher WMH burden, and greater hypoperfusion in regions susceptible to changes in AD (temporal cortex, angular gyrus and posterior cingulate gyrus) and VaD (frontal pole and anterior cingulate cortex).

Study Aim 2 We will compare amnestic and non-amnestic subtypes of MCI using neuroimaging biomarkers of β-amyloid, white matter lesion and cortical and regional cerebral perfusion.

Hypothesis 2 We hypothesize that amnestic MCI patients will have higher PiB retention, lower WMH burden and reduced perfusion in the temporal cortex, angular gyrus and posterior cingulate gyrus compared to non-amnestic MCI. In contrast, non-amnestic MCI will have lower PiB retention, higher WMH burden and reduced perfusion in the frontal pole and anterior cingulate cortex when compared to amnestic MCI.

Study Aim 3 We will determine the relationship between cortical and regional cerebral perfusion and 1) PIB retention, and 2) WMH burden in our study cohort consisted of SCC and MCI patients.

Hypothesis 3 We hypothesize that higher PiB retention will be correlated with hypoperfusion in regions susceptible to changes in AD, including the temporal cortex, angular gyrus and posterior cingulate cortex. Increased WMH burden will be correlated with hypoperfusion in the frontal pole and anterior cingulate cortex.

1.7 Contributions to this Project

I was responsible for formulating a hypothesis driven research question for this Master's project using data previously collected as part of a prospective longitudinal cohort study co-led by thesis supervisor Dr. Smith and collaborators at the Massachusetts General Hospital Alzheimer's Disease Research Centre (ADRC). As such, participant recruitment and neuroimaging and neuropsychological assessment data collection were completed in Boston, Massachusetts. The assessment of cognitive status and categorization of patients, based on clinician's impression and neuropsychological testing, was completed in collaboration with Dr. Deborah Blacker, a geriatric psychiatrist. Although I was not able to participate in this off-site process, I have participated in similar neuropsychological assessments at the Cognitive Neuroscience Clinic at the University of Calgary. The PiB-PET data was collected and analyzed by Dr. Keith Johnson of Massachusetts General Hospital. Dr. Eric Smith and Karla Sanchez, a previous research assistant in the Smith lab, were responsible for deriving WMH volume from T2-FLAIR images collected at the ADRC. Although WMH calculations were completed when I started my Master's degree, I was still trained by Dr. Smith on how to use the software (Quantomo, Cybertrial, Calgary, AB) to

determine WMH volume in other studies conducted in our laboratory. I was responsible for processing the ASL perfusion data obtained from our collaborators at the ADRC, using software (FSL, Oxford University, Oxford) to make quantitative calculations of cerebral blood perfusion. With a complete set of processed neuropsychological and neuroimaging data, I was responsible for conducting univariate and power analyses found in this thesis while Dr. Smith taught and supervised the multivariable analyses.

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Chapter Two: **METHODS**

2.1 Study Population

To address our study aims (Chapter 1) we retrospectively analyzed data from 61 eligible subjects who participated in a prospective longitudinal cohort study co-led by thesis supervisor Dr. Smith and collaborators at the Massachusetts General Hospital Alzheimer's Disease Research Centre (ADRC). The three objectives of this cohort study were: 1) To determine whether increased whole-brain proton diffusivity is a predictor of future cognitive decline, 2) To determine whether white matter lesions in specific regions can cause cognitive decline in specific domains, and 3) To determine whether white matter lesions are associated with cognitive decline, independent of β-amyloid accumulation.

Participants were recruited from a memory clinic at the Massachusetts General Hospital ADRC. The inclusion criteria were: >60 years old, Clinical Dementia Rating (CDR) score of 0.5, subjective cognitive concerns by the patient or caregiver/close informant, and history of hypertension. The CDR is a 5-point rating system commonly used for a global assessment of cognitive impairment, which ranges from normal to severe dementia (Morris *et al*., 1993). CDR 0.5 corresponds to "questionable dementia", but without a confirmed dementia diagnosis. There are six different components to the CDR, including memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. History of hypertension was selected as an inclusion criterion in order to enrich the study cohort with patients with a larger burden of white matter hyperintensity (WMH) of presumed vascular origin (Wardlaw *et al*., 2013), a pre-specified exposure of interest.

Participants were excluded from the parent study if they were diagnosed with dementia based on the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria

(American Psychiatric Association. and American Psychiatric Association. Task Force on DSM-IV., 1994), had a history of symptomatic stroke, were diagnosed with causes of cognitive impairment other than possible AD or vascular cognitive impairment, had diabetes mellitus requiring insulin, had any other central nervous system disease, psychiatric disorders or psychosis other than stable treated depression, or had MR contraindications. All participants in the retrospective analyses were also required to have completed all three neuroimaging sequences, including PiB-PET, T2-FLAIR and ASL MR imaging.

2.2 Neuropsychological Assessments

Neuropsychological assessments were administered according to the National Alzheimer's Coordinating Center Uniform Data Set (Beekly *et al*., 2007). Routine Mini Mental State Examination (MMSE) were also obtained and provided a psychometric assessment of cognitive impairment, and assessed five areas of cognition based on eleven questions, including orientation, registration, attention and calculation, and recall and language (Folstein *et al*., 1975). In a highly educated population, cut-off score of 27 out of 30 yielded 69% sensitivity and 91% specificity for detecting cognitive impairment in MCI and dementia compared to healthy controls (O'Bryant *et al*., 2008).

We assessed three cognitive domains that are most commonly impaired in AD and vascular dementia including episodic memory, executive function and processing speed. The *z* score of each cognitive domain was a calculated average of two neuropsychological assessments for each domain.

The Wechsler Logical Memory II and California Verbal Learning Test II (CVLT-II) Long Delay Free Recall tests were used to assess episodic memory. During the Wechsler Logical

Memory II assessment, two passages are read by the examiner to the participant, and he/she will be asked to recall the material twice, once immediately and another time after a delay (Weschler 1987). In the CVLT-II, participants were asked to undergo five learning trials of 16 words and once completed, another word list will be presented for a single learning trial (Delis *et al*., 2005). From the total of six primary measures of the test–including trials 1-5 total learning, short-delay free recall, short-delay cued recall, long-delay free recall and total recognition discrimination–long-delay free recall was selected because it is the best method of identifying MCI patients likely to progress to dementia (Molinuevo *et al*., 2011).

The *z* score representing executive function was an average of verbal fluency (including animal and vegetable naming semantic fluency and letter fluency), and Trail Making B minus Trail Making A. The Controlled Oral Word Association Test (COWAT) was used to assess both semantic and letter fluency (Benton *et al*., 1994). To assess semantic fluency, participants were asked to give examples of as many animals or vegetables within 60-s trials. To assess letter fluency, participants were asked to generate as many words as possible, within 60-s, that begin with the letters "F", "A" and "S" on three consecutive trails. The Trail Making Test (TMT) consists of a two-part psychomotor task to assess executive function (Reitan 1986). In TMT-A, participants are asked to connect a line as fast as possible between circles (numbered 1 to 25) that are scattered in a pre-determined pattern on the page. TMT-B asked the participant to perform a similar task, but this time alternating between letters (from A to L) and numbers (from 1 to 13). Subtracting the TMT-A scores from TMT-B provides a good measure of cognitive flexibility that is independent of manual dexterity (Corrigan and Hinkeldey 1987). The TMT-B minus TMT-A scores were right-skewed, and therefore logarithmically transformed so that the scores were more normally distributed.

Processing speed function was determined using the TMT-A and Digit Symbol Substitution (DSS) tests (Weschler 2008). The TMT-A is described in the preceding paragraph. During the DSS test, which is a subtest of the Wechsler Adult Intelligence Scale (WAIS), the subjects were asked to code a list of digits with reference to a digit-symbol pair list provided (*i.e.*, 1/-,2/ ⊥).

2.3 Categorization of SCC, MCI and MCI Subtypes

An experienced dementia clinician (Deborah Blacker) reviewed the CDR scores, neuropsychological test scores, participant demographics and past medical history to assign SCC or MCI status to each patient, blinded to neuroimaging results. The NIA-AA criteria (Albert *et al*., 2011) were used to distinguish MCI from SCC, and categorization of MCI subtypes were determined by domain-specific impairments on the CDR and neuropsychological testing. As described earlier, episodic memory, executive function and processing speed were examined. Neuropsychological test scores were converted to individual *z* scores based on a normative database, which consists of data from 303 asymptomatic participants at the Massachusetts General ADRC. A *z* score lower than 1.0 standard deviation (SD) was considered impairment relative to the cognitively healthy population.

Participants were assigned SCC status if they scored above -1.0 SD on neuropsychological tests for episodic memory, executive function and processing speed. Patients with MCI were further subcategorized as either amnestic or non-amnestic. Amnestic MCI was defined as MCI with episodic memory domain score less than 1.0 SD below the normative mean, with or without evidence of impairment in executive function or processing speed. The remaining MCI patients were classified as non-amnestic MCI, meaning that they had scored less

than 1.0 SD below the normative mean on either or both of executive function or processing speed, but with normal range performance on episodic memory.

2.4 Neuroimaging Biomarkers and Techniques

2.4.1 PiB-PET measurement of β-amyloid

PiB-PET was used in the study to quantify β-amyloid accumulation in the cerebral cortex. β-amyloid plaques found in the brain have been long recognized as the clinical hallmark of AD. PiB is a derivative of thioflavin-T, binding specifically to fibrillary β-amyloid deposits and ^{11}C is the radioisotope that allows for detection using PET (Klunk *et al*., 2004). Due to the short half-life of ^{11}C (20 minutes), research using PiB-PET is limited to sites with a cyclotron. PiB-PET imaging is an attractive technique because it allows *in vivo* measurement of β-amyloid pathology and quantification based on regional analysis (Figure 2-1). The distribution volume ratio (DVR) is often used as a method of quantifying β-amyloid, such that the cerebellar cortex, with no specific binding for PiB, is used as reference tissue (Logan 2000). Using PiB-PET, we will have a better understanding of the contribution of cortical β-amyloid in distinguishing SCC from the MCI population (Aim 1), neuropathology underlying amnestic and non-amnestic MCI (Aim 2) and the relationship between β-amyloid and regional cerebral perfusion in a population with early cognitive decline (Aim 3).

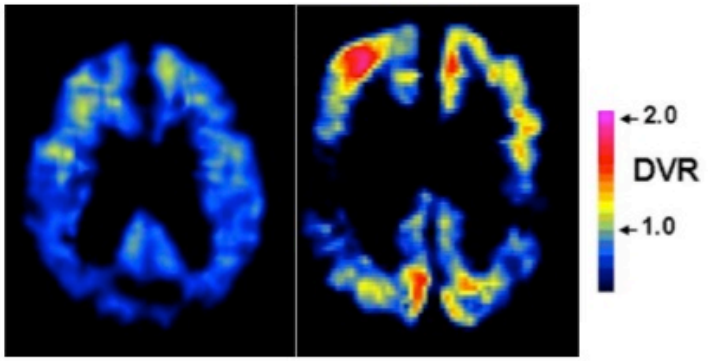

Figure 2-1 Axial slices from two patients showing varying levels of PiB distribution volume ratio (DVR) in PET imaging, which is a quantitative measurement of β-amyloid accumulation.

2.4.2 T2-FLAIR MR measurement of White Matter Hyperintensity

Cerebral small vessel disease (SVD) is the most common vascular cause of dementia, which causes approximately 20% of all dementia cases, second in prevalence only to AD. White matter lesions are the most common manifestation of SVD. They are visible as hyperintense signal on T2 Fluid Attenuated Inversion Recovery (FLAIR) MR imaging (Figure 2-2). When visualized on MR imaging, white matter lesions may also be referred to as white matter hyperintensity (WMH) of presumed vascular origin (Wardlaw *et al*., 2013). White matter lesions manifest as hyperintense signals on T2-FLAIR images because they have longer T2 relaxation rates compared to healthy brain tissue, due to the higher water content and structural degeneration within the lesions. Amongst T2-weighted spin echo sequences and proton density, both of which are able to detect WM signal changes, T2-FLAIR is superior for detecting periventricular WMH, because in T2-FLAIR, an inversion pulse nulls the cerebrospinal fluid (CSF) signal, allowing for discrimination of periventricular WMH from CSF (Rovaris *et al*. 1999; Rydberg *et al*. 1995). By

quantifying the degree of WMH burden, we will have a better understanding of how it contributes to distinguishing SCC from MCI (Aim 1), neuropathology underlying amnestic and non-amnestic MCI (Aim 2), and the relationship between WMH burden and regional cerebral perfusion (Aim 3).

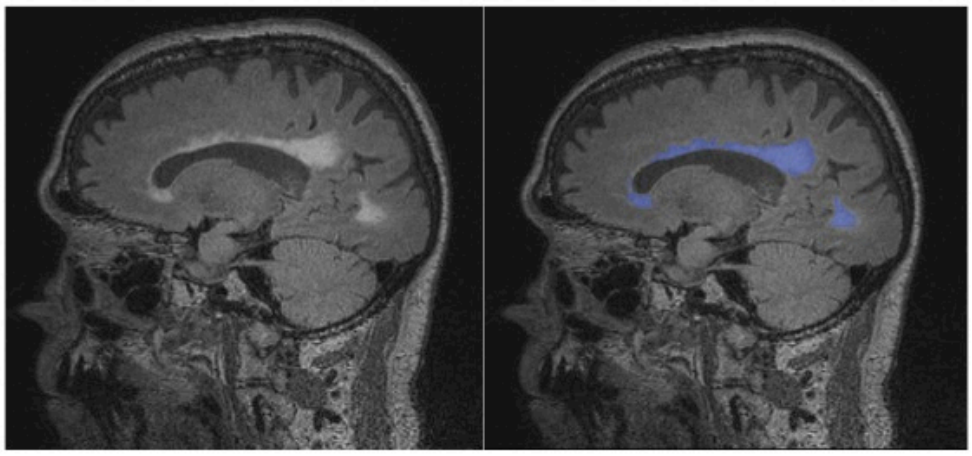

Figure 2-2 Sagittal slice of T2-FLAIR showing white matter hyperintensity (left). The blue mask overlying WMH (right) is created in Quantomo (Cybertrial, Inc: Calgary, Canada), which calculates a lesion volume based on the marked boundaries.

2.4.3 Arterial Spin Labeling MR measurement of Cerebral Perfusion

Arterial spin labeling (ASL) is one of the first non-invasive and *in vivo* MR techniques to measure tissue perfusion (Figure 2-3). Although $H_2{}^{15}O$ Positron Emission Tomography is the gold standard for quantitative assessment of cerebral perfusion, studies have validated ASL against this method (Carroll et al., 2002). In ASL, arterial blood water protons are inverted using a radiofrequency (RF) pulse to create an endogenous tracer, and labeled images are acquired (Edelman *et al*., 1994). Control images are also acquired during image acquisition, and cerebral blood flow (CBF) maps are generated after subtracting the labeled images from the control

images to any eliminate static tissue signal. There are different types of ASL techniques that differ primarily based on the magnetic labeling process. This study employs the use of pulsed ASL (PASL), which is known for its labeling efficiency (Chen *et al*., 2011). In PASL, the non-selective short RF pulse (5-20ms) is applied to a labeling region upstream of the imaging region. Using ASL, we will have a better understanding of differences in cerebral perfusion of key regions affected in dementia within our SCC and MCI cohorts (Aim 1 and 2), and we will also be able to correlate absolute measurements of cerebral perfusion to quantitative measurements of neuropathology, *i.e*., β-amyloid accumulation and WMH burden (Aim 3).

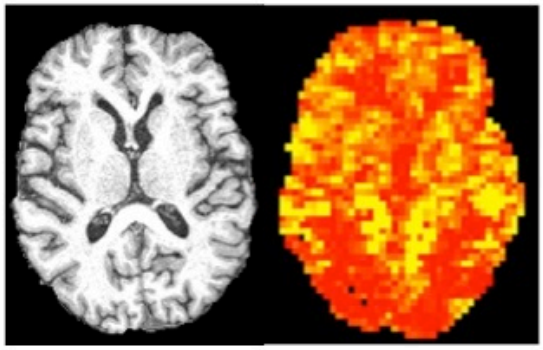

Figure 2-3 Axial slices of high-resolution T1-weighted brain and ASL derived cerebral blood flow map used for cerebral perfusion measurements (mL/100g/min).

2.5 Neuroimaging Parameters and Processing

All neuroimaging data were collected at and in collaboration with Massachusetts General Hospital Alzheimer's Disease Research Centre.

2.5.1 Pittsburgh Compound B PET

Mean cortical PiB-PET was acquired in 3D mode on the Siemens/CTI ECAT HR+ scanner (Siemens CTI, Knoxville, TN) using the following parameters: 63 image planes, 15.2 cm axial field-of-view, 5.6 mm trans-axial resolution and 2.4 mm slice interval, 69 frames: 12x15 seconds, 57x60 seconds. ^{11}C-PiB was prepared according to the standard method described by Mathis *et al*. (2003) and 8.5 to 15mCi was injected intravenously as a bolus into the antecubital vein. The dynamic acquisition of PiB-PET data was 60 min.

Each frame of PET data was evaluated by Dr. Keith Johnson, an experienced neuroradiologist, to verify adequate count statistics and the absence of head motion. The distribution volume ratio (DVR), which uses the cerebellum as reference tissue, was used to quantify β-amyloid burden in the cortical grey matter regions (Lopresti *et al*., 2005, Price *et al*., 2005). The DVR is derived using the Logan graphical analysis, which is based on compartmental equations used to describe PiB accumulations in the tissue (Logan 2000). Multiple time measurements within the plasma and tissue were transformed into a linear plot, where the slope was related to the number of available tracer binding sites. Post-acquisition processing of PET data was also completed off-site by our collaborators at ADRC. As in previous studies (Rentz *et al*., 2010), PiB DVR value of 1.25 was used to dichotomize patients as either PiB+ or PiB-.

2.5.2 White Matter Hyperintensity on T2-FLAIR

T2-FLAIR were acquired on a 3.0 T Siemens Trim Trio MR (Siemens Medical Systems, Erlangen, Germany) to identify WMH. The MR protocol was the following: TR/TE/TI/α = 6000ms/ 455ms/2100ms/120°, FOV = 256mm, matrix = 256 x 254, 176 slices with 1mm slice thickness, and a bandwidth of 698 Hz/pixel.

WMH were defined according to the STandards for Reporting Vascular Changes in nEuroimaging (STRIVE) criteria (Wardlaw *et al*., 2013). Volumetric WMH analysis was completed using Quantomo (Cybertrial, Inc: Calgary, Canada), a custom-designed software developed at the University of Calgary. Quantomo operates on a semi-automated basis, which enables certified raters to select areas of hyperintensity on T2-FLAIR in both periventricular and subcortical white matter regions. The lesion volume, measured in mL, was calculated for each subject based on the software's seed-growing algorithm. In order to account for differences in patient head size, WMH volumes will be expressed as a percentage of intracranial volume. Logarithmic transformation will be applied to report findings because WMH volume distribution is highly right-skewed.

2.5.3 Arterial Spin Labeling Cerebral Perfusion

PASL images were acquired on a 3.0 T Siemens Trim Trio MR scanner (Siemens Medical Systems, Erlangen, Germany) using the following parameters: TR/TE/α = 4000 ms/13 ms/90°; matrix = 64 x 64; FOV 320 mm, 24 slices with slice thickness 4 mm and 5 mm interslice gap; bandwidth 2298 Hz/pixel. Using FMRIB Software Library (FSL, Oxford University, Oxford), the labelled images were subtracted from the control images to obtain a CBF map for each of the participants.

Skull stripping and extraction of the control ASL images were performed and subsequently co-registered to a high-resolution anatomical T1-image of the subject's brain (MP-RAGE: TR/TE/α = 2300 ms/ 2.98ms/9°; matrix = 256 x 240; FOV 256 mm; 176 slices with 1mm slice thickness; bandwidth 238 Hz/pixel). Following co-registration, CBF images were transformed into Montreal Neurological Institute (MNI) space, and MNI-152 structural templates

(Maintz and Viergever) were applied to identify the regions of interest (ROI). Regional cerebral perfusion values were calculated using FSL for each patient. Since the reliability of white matter perfusion derived from PASL has been criticized in literature because of low signal to noise to ratio (van Gelderen *et al*., 2008), we relied on grey matter perfusion to understand the global cerebral perfusion. We created a binary mask of the grey matter (GM) segmented from the patient-derived T1-weighted images, which was applied to the whole brain to calculate perfusion only in the GM.

The focus of the thesis is to investigate perfusion of regions most susceptible to changes in dementia. To determine our regions of interest (ROI), we referenced a meta-analysis published by Landau *et al*. (2011) that describes regions that are most susceptible to FDG-PET hypometabolism in AD and MCI (Figure 2-4). Using 209 cross-sectional/correlational studies and 31 longitudinal studies, voxel-wise analyses showed that regions within the bilateral middle/inferior temporal gyrus, bilateral angular gyrus and bilateral posterior cingulate gyrus were more susceptible to hypometabolism in AD and MCI. In another study, these anatomical regions were also shown to exhibit hypoperfusion in AD (Wang *et al*., 2013).

To investigate perfusion changes related to WMH, we also included regions that we hypothesized would reflect changes corresponding to subcortical vascular injury (Kerrouche *et al*., 2006). Frontal lobe dysfunction is proposed to be a key component of vascular dementia. Therefore, we chose *a priori* to examine the frontal pole and anterior cingulate cortex, as outlined by the Harvard-Oxford Atlas in FSL. Damage to these anatomical regions could result in impairment in executive function, which is the most common cognitive impairment in vascular dementia (Roh and Lee 2014).

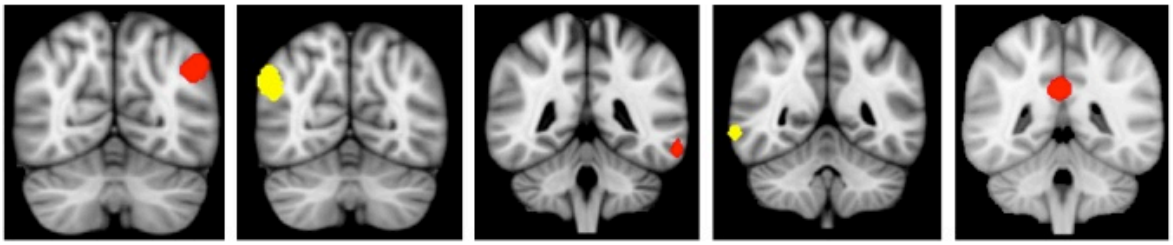

Figure 2-4 The five regions of AD-ROI (Landau *et al.*, 2011) are masked onto the MNI T1 brain. From left to right: left angular gyrus, right angular gyrus, left temporal gyrus, right temporal gyrus and posterior cingulate cortex

2.6 Statistical Analyses

To test our three hypotheses, both univariate statistical analyses and multivariable linear regression models were applied where appropriate. In Aims 1 and 2, the comparisons of β-amyloid accumulation, WMH burden and ASL perfusion between each cohort were completed using the independent two-sample *t*-test. To test differences in sex and other dichotomous variables between groups, Fischer's exact test was used. To determine the relationship between PiB DVR or WMH burden and perfusion in our ROIs as a part of Aim 3, we used the Pearson correlation coefficient. The multivariable analyses were used to adjust for any differences due to age in Aim 3. All univariate analyses were completed using commercial software (GraphPad Prism version 6; GraphPad Software Inc., La Jolla, CA, USA) and all multivariable analyses were completed in collaboration with Dr. Eric Smith (SAS version 9.3; SAS Institute, Cary, NC, USA). A p-value < 0.05 was considered statistically significant. Power analyses in Aims 1 and 3 were conducted using G*Power (Franz Faul, Universitat Kiel, Germany).

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Chapter Three: **NEUROIMAGING BIOMARKERS OF DEMENTIA IN PATIENTS WITH SUBJECTIVE COGNITIVE CONCERNS *VS.* MILD COGNITIVE IMPAIRMENT**

3.1 Introduction

Patients with subjective cognitive concerns (SCC) account for a significant portion of visits to cognitive clinics worldwide (Wahlund *et al*., 2003), with the majority expressing perceived memory concerns. The concept of SCC is currently a hot debate in dementia research. While some researchers believe that SCC is an early sign of pathological cognitive decline, others argue that SCC primarily reflects non-organic causes such as co-existing mood disorders (Reisberg *et al*., 2008; Abdulrab *et al*., 2008). Since it is a relatively new concept in the field of dementia, there was initially a lack of consensus on the terminology used to describe this concept. For example, subjective cognitive impairment (SCI), subjective cognitive decline (SCD) and pre-MCI are all synonyms of SCC. Terms such as subjective memory impairment (SMI) have been used to describe subjective concerns with memory, which could also be considered a sub-classification of SCC.

It is estimated that AD pathology appears 10-20 years prior to a clinical diagnosis of MCI or AD (Jack *et al*., 2010), and therefore could possibly account for many cases of SCC. Many studies are trying to understand whether SCC is a risk factor for progression to MCI and to dementia, which patients will progress, and what type of dementia (i.e. AD, vascular or other) that they are at risk for.

There are many causes of SCC including AD/prodromal AD, psychiatric and neurological disorders unrelated to AD including cerebrovascular disease, and effects of medication and substance abuse. Since Alzheimer's disease and cerebrovascular disease account for the majority of dementia cases, it is logical to investigate the prevalence of such pathology in

SCC as well. Therefore we chose to investigate β-amyloid accumulation and white matter lesion burden, along with cortical and regional cerebral perfusion biomarkers.

Studies so far have reported inconsistent results on the relationship between SCC and AD pathology. Since β-amyloid deposits are thought of as antecedent signs of AD-dementia, a key focus of SCC research is detecting amyloidosis. PiB-PET imaging and CSF biomarkers of AD have been studied in the SCC population and compared to healthy aging and MCI or AD (Rodda *et al*., 2010; Chetelat *et al*., 2010; Verdil *et al*., 2014; Ivanoiu *et al*., 2015). These studies report increased β-amyloid accumulation with disease progression, though to various extents. Correlational studies also show that increased β-amyloid deposits are associated with subjective complaints of memory and executive function in cognitively healthy adults (Amariglio *et al*., 2012). Studies that dichotomized cognitively healthy adults with subjective complaints into PiB-positive and PiB-negative reported inconsistent findings on the extent of cognitive impairment in each group. While PiB positivity was found in some studies to be associated with greater memory and executive function impairment (Amariglio *et al*., 2012; Perrotin *et al*., 2012), other studies failed to establish the same conclusion (Hollands *et al*., 2015).

Much like the evolution of AD, underlying small vessel disease also builds up over a number of years, eventually resulting in brain lesions such as WMH and infarction. Thus, we were also interested in examining the extent of WMH damage in SCC compared to MCI. The role of WMH within the SCC population has been poorly defined according to current literature, although many studies have described key findings within healthy aging and MCI cohorts. It has been shown that white matter lesions due to small vessel disease contribute to the rate of cognitive decline during the earlier stages of neurodegeneration (Tullberg *et al*., 2004; Schneider *et al*., 2004). Cerebrovascular contributions to cognitive decline can often be found within the

presence of AD pathophysiology. Vemuri *et al*. (2015) demonstrated that though there is a lack of synergistic effect of cerebrovascular disease and AD pathology on cognitive decline, the effect may be additive. This conclusion is supported by findings that faster rate of cognitive decline is present in those with both AD and cerebrovascular disease (Del Ser *et al*., 2005).

Because most of the recent cerebral perfusion imaging works focus on MCI and AD stages of dementia, there is a paucity of literature focused on SCC. Hypoperfusion have consistently been reported in the bilateral parietal cortex, precuneus and posterior cingulate cortex of AD patients, but to a milder extent in MCI (Binnewijzend *et al*., 2013; Dai *et al*., 2009; Johnson *et al*., 2005). Binnewijzend *et al*. (2015) showed greater hypoperfusion in the temporoparietal regions of dementia patients compared to predementia (consisted of both preclinical AD and MCI) patients with abnormal CSF Aβ but normal CSF tau. This study was among the first to compare ASL perfusion in predementia patients, categorized based on CSF AD biomarkers.

In this chapter, mean cortical PiB, WMH burden and cortical and regional cerebral perfusion are compared in patients with SCC and MCI. We hypothesized that since SCC represents an earlier stage of cognitive decline than MCI, therefore, patients with SCC will exhibit lower mean cortical PiB retention, lower WMH burden, less cortical and regional hypoperfusion in regions affected by AD and VaD compared to SCC.

3.2 Results

3.2.1 Subject Characteristics of SCC and MCI

The subjects were dichotomized into SCC or MCI cohort based on *z* scores of episodic memory, executive function and processing speed as described in Chapter 2. Based on previously

described criteria, 43 patients qualified as SCC and 18 patients qualified as MCI. As shown in Table 3-1, the average age between patients with MCI (74.1 ± 8.5 years) and SCC (75.1 ± 6.8 years) did not differ significantly, $p = 0.64$. There were 50% and 49% females in the MCI and SCC cohorts, respectively. Overall, patients with SCC had higher level of education (16.7 ± 2.4 years) compared to the patients with MCI (15.2 ± 2.4 years), $p < 0.05$.

In order to assess for possible confounders to our neuroimaging measurements, we also compared smoking status, diabetes, hypertension, hypercholesterolemia, history of depression, presence of silent brain infarcts, lobar and deep cerebral microbleeds between the two cohorts. There were no significant differences between these clinical and neuroimaging measures (Table 3-1).

Table 3-1 Subject characteristics of SCC and MCI

Variable	**SCC** n = 43	**MCI** n = 18	***p*-value**
Age (years±SD)	75.1 ± 6.8	74.1 ± 8.5	0.64
Sex (% female)	48.8	50.0	1.00
Education (years ±SD)	**16.7 ± 2.4**	**15.2 ± 2.4**	**0.02**
MMSE (±SD)	**28.8 ± 1.2**	**28.0 ± 1.5**	**0.02**
Clinical Profile (n)			
Current smoker	5 (12%)	1 (5.5%)	0.66
Diabetes	7 (16%)	3 (17%)	1.00
Hypertension	42 (98%)	18 (100%)	1.00
Hypercholesterolemia	32 (74%)	10 (56%)	0.22
Depression (prior diagnosis)	15 (35%)	9 (50%)	0.39
Depression (within 2 years)	13 (30%)	8 (44%)	0.38
Silent brain infarcts	7 (16%)	2 (11%)	0.71
Lobar CMB	11 (26%)	1 (5.5%)	0.09
Deep CMB	2 (4.7%)	1 (5.5%)	1.00
Neuropsychological Profile (*z* score)			
Executive function	**0.03**	**-1.08**	**<0.0001**
Episodic memory	**-0.10**	**-1.66**	**<0.0001**
Processing speed	**-0.02**	**-0.54**	**0.017**

Key – MMSE = Mini-Mental Status Examination, CMB = cerebral microbleeds

3.2.2 Neuropsychological Assessments

Neuropsychological assessments revealed significant cognitive function differences between SCC and MCI, as expected. The global assessment of cognitive function revealed that MCI scored lower on the MMSE (28.0 ± 1.5) compared to SCC (28.8 ± 1.2), $p < 0.02$. Consistent with the SCC/MCI classification criteria, the SCC cohort scored above -1.0 SD in all cognitive domains, including episodic memory ($z = -0.10$), executive function ($z = 0.03$) and processing speed ($z = -0.02$). The MCI cohort scored below the cut-off in episodic memory ($z = -1.66$) and

executive function (z = -1.08) but not in processing speed (z = -0.54). As shown in Table 3-1, SCC still scored significantly higher than MCI in all cognitive domains assessed.

3.2.3 Neuroimaging Biomarker Characteristics

PiB retention did not differ between MCI (1.30 ± 0.25) and SCC (1.22 ± 0.20), $p = 0.23$. Based DVR of 1.25 as cut-off, 26% and 44% of SCC and MCI patients, respectively, were PiB-positive, although this difference was not significant ($p = 0.22$). There were also no significant differences in cerebral perfusion between our ROIs, as displayed in Table 3-2. However, SCC patients had higher median log percentage WMH volume (-0.57, IQR -0.95 to -0.17) compared to the MCI patients (-0.90, IQR -1.38 to -0.46), $p = 0.003$. After adjusting for education, we still found higher log percentage WMH in SCC compared to MCI, $p = 0.01$.

Power analyses done using G*Power determined that our sample size of 43 SCC and 18 MCI patients will have 80% power to detect a 0.80 SD difference in neuroimaging parameters.

Table 3-2 Neuroimaging biomarker characteristics of SCC and MCI

Variable	**SCC** n = 43	**MCI** n = 18	***p*-value**
PiB DVR (±SD)	1.22 ± 0.20	1.30 ± 0.25	0.23
PiB+ (DVR >1.25)	11 (26%)	8 (44%)	0.22
% WMH (IQR)	**0.27 (0.11to 0.68)**	**0.13 (0.04 to 0.36)**	**0.02**
Log % WMH (IQR)	**-0.57 (-0.95 to -0.17)**	**-0.90 (-1.38 to -0.46)**	**0.006**
Arterial Spin Labeling Perfusion (mL/100g/min, (±SD)			
Grey Matter	45.6 ± 6.4	46.5 ± 4.7	0.60
AD-ROI	51.3 ± 10.3	50.3 ± 6.7	0.35
L Angular Gyrus	51.8 ± 10.3	49.7 ± 9.5	0.44
R Angular Gyrus	49.7 ± 11.5	49.0 ± 5.5	0.81
L Temporal Gyrus	46.9 ± 12.9	44.5 ± 12.9	0.50
R Temporal Gyrus	46.6 ± 13.0	45.2 ± 10.1	0.67
Posterior Cingulate Cortex	55.4 ± 15.3	54.5 ± 10.9	0.82
Anterior Cingulate Cortex	45.6 ± 7.5	45.0 ± 6.2	0.76
Frontal Pole	46.6 ± 7.9	49.0 ± 7.6	0.28

Key – WMH = white matter hyperintensity, IQR = interquartile range, ROI = region of interest, L = left, R = right

3.3 Discussion

Overall, there was comparable PiB retention and cerebral perfusion in SCC and MCI, but higher WMH burden was found in the SCC cohort.

3.3.1 Pittsburgh Compound-B Retention

The MCI cohort in our study had higher PiB (DVR, 1.30 ± 0.06) compared to SCC (DVR, 1.22 ± 0.03), but the difference was not significant. Similarly, although 44% of MCI were PiB positive compared to only 26% of SCC, this difference was not significant. Our findings are contrary to

existing literature that found differences in cortical PiB retention between SCC and MCI (Chetelat *et al*., 2010; Verdile *et al*., 2014; Ivanoiu *et al*., 2015).

Without a cognitively healthy group for comparison, we cannot make conclusions based on the degree of β-amyloid accumulation relative to the healthy population. However, it is still worth noting that our SCC cohort has accumulated a sufficient amount of β-amyloid, even when compared to the MCI cohort. Within our highly educated cohort, we cannot disregard the effect of high-cognitive reserve, assessed by years of education, on the categorization of SCC (Vemuri *et al*., 2012; Wilson *et al*., 2013). It is possible that even in the presence of β-amyloid accumulation sufficient to cause cognitive impairment, patients with SCC in our cohort are less susceptible to the effects of β-amyloid.

Overall, the lack of significant β-amyloid retention differences between the SCC and MCI cohorts may still add validity to the concept proposed by Donovan *et al*. (2014), where SCC is considered a milder case of predementia relative to MCI. Due to the arbitrary nature of commonly used neuropsychological performance cut-offs (*i.e*., -1.0 SD or -1.5 SD), it is possible that by categorizing participants based on these cut-offs, we are essentially separating very mild cases of MCI from very severe cases of SCC, such that the MCI and SCC cohorts may not be that different in terms of objective cognitive impairment. Thus, future experiments should also examine the relationship of β-amyloid levels on neuropsychological performance within each cohort. An alternative explanation for the lack of significant β-amyloid finding could be due to the heterogeneous nature of both cohorts. The clinical utility of MCI subtypes has been previously highlighted, since unique characteristics of each subtype are often masked when studied as a whole cohort (Winblad *et al*., 2004). Since SCC represents a very diverse population

of patients presenting to the clinic, future studies on the best method for subtyping may be worthwhile.

3.3.2 White Matter Hyperintensity Burden

I was surprised to observe more WMH in our SCC cohort than our MCI cohort, contrary to our pre-specified hypothesis. We can disregard the potentially confounding effect of age, a known predictor of WMH prevalence, and degree of WMH burden in our cohort (de Leeuw *et al*., 2001), since age did not differ significantly between SCC and MCI. WMH burden in our cohort was higher than in similar-aged cognitively normal participants in the Framingham Heart Study (Au *et al*., 2006), probably as a result of the inclusion criterion of history of hypertension.

A potential explanation for the difference in WMH between SCC and MCI may be that WMH is sufficient to cause mild impairment and subjective concerns, but typically not sufficient to cause the more severe objective evidence of impairment seen in MCI. In healthy cohorts, higher volume of WMH is associated with worse performance on cognitive testing (Au *et al*., 2006). However, the degree of impairment is relatively mild. Thus, higher WMH volumes may contribute to subjective perception of a memory concern, but without causing objective cognitive impairment defined as performance less than 1.0 SD below the mean. If this is true, then there must be alternative non-WMH related factors that contribute to the risk for MCI.

Future research studies should conduct a longitudinal follow-up of this SCC cohort with higher WMH burden, and assess whether they proceed at a faster annual rate of cognitive decline in executive function or conversion to non-amnestic MCI.

3.3.3 Arterial Spin Labeling Cerebral Perfusion

The biomarkers cascade model, proposed by Jack *et al*. (2010), suggests that upstream β-amyloid accumulation leads to neurodegeneration. It has been estimated that neuronal injury can occur up to 20-30 years prior to onset of symptoms. While many research studies focused on using FDG-PET as the primary method of detecting neuronal injury, fewer studies have used ASL perfusion. Since regional cerebral perfusion and glucose metabolism are coupled (Raichle *et al*., 1998), ASL and FDG-PET serve as good alternatives for visualizing and quantifying changes in cerebral and neuronal functions (Musiek *et al*. 2012).

Without a cognitively healthy group for comparison, it is difficult to ascertain relative changes in cerebral perfusion. Contrary to what was expected, we did not detect any significant perfusion differences in our ROIs (regions known to be susceptible to pathology in dementia), between SCC and MCI. This finding may indicate a relative lack of difference between SCC and early stage MCI. A study that staged MCI, based on the severity of memory impairment, found that in less severe memory impairment, there was a lack of FDG-PET hypometabolism even in the presence of β-amyloid (Wu *et al*., 2012). Similarly, Li *et al*. (2008) found that only 54% of the PiB-positive MCI patients showed FDG-PET hypometabolism, even after considering the most sensitive regions for analysis. Therefore, our findings combined with others suggest that cognitive symptoms can occur prior to emergence of hypoperfusion due to AD. We cannot comment on the extent of hypoperfusion relative to unimpaired persons, due to the absence of a control group in our study. Future studies should focus on understanding the relationship between hypoperfusion and β-amyloid in early stages of cognitive decline including SCC and MCI.

Despite higher WMH burden in SCC, we did not find a lower perfusion in SCC *vs*. MCI globally or in the frontal brain regions we hypothesized would be most affected by hypoperfusion due to WMH. Analyses of the relationships between PiB-PET, WMH and ASL perfusion, pooling SCC and MCI together, are described in Chapter 5.

3.3.4 Study Limitations

There are a few important limitations to our study design that prevent us from drawing more conclusions. First, without a cognitively healthy group for comparison, conclusions about neuroimaging biomarker values in SCC and MCI can be made relative to each other but not in reference to values within the healthy aging population. The addition of a healthy age- and sex-matched group would address this limitation. Second, a larger sample size would have helped to draw more meaningful conclusions from two cohorts with heterogeneous disease processes. Third, our study design categorized patients as SCC *vs*. MCI based on consensus-derived neuropsychological test score cut-offs. Assuming transition of SCC to MCI is part of a continuum, these cut-off values become arbitrary. In a study with a larger sample size, correlational analyses of biomarkers with neuropsychological test performance within SCC and MCI could address this issue. Lastly, since APOE ε4 genotyping was not available as a part of this retrospective research question, we were unable to account for its possible effects on neuroimaging biomarker findings and cognitive complaint.

3.3.5 Conclusions and Future Directions

SCC probably results from a heterogeneous group of processes, requiring further research. Collectively, research on SCC subtypes is in a relatively early phase. Therefore, we suspect that

sub-classification of SCC, based on future longitudinal research studies, may prove to be similarly useful for prognostication of SCC outcomes.

With the lack of disease modifying medications for AD, the implications of earlier diagnosis are still uncertain. Expert opinions suggest that early detection of cognitive decline can optimize medical management, offer relief based on understanding the symptoms, autonomy and planning for the future. One approach would be to develop more sensitive, new neuropsychological measures. According to current standard neuropsychological tests, SCC are "normal" but a subgroup probably harbour very early pathological changes and are destined to develop progressive cognitive decline over time. Our study is among the first to simultaneously utilize three neuroimaging biomarkers to understand how SCC differs from MCI, and this is important not only for elucidating underlying pathophysiological mechanisms of SCC, but also could be important for implementing management strategies that may be more effective in early stages of cognitive decline.

For future studies, it would be worthwhile to investigate the dynamics of neuroimaging biomarkers in SCC over longitudinal follow-up, in order to gain a better understanding of disease progression. For example, it would be interesting to determine the progression rate to vascular dementia in a SCC population with higher WMH burden. We can also hope to increase sensitivity and specificity for predicting progression by combining neuroimaging biomarkers with CSF biomarkers. Effectively treating patients with SCC destined for future dementia would preserve function at a higher level before progressive neuronal loss and irreversible damage occur.

3.4 References

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Chapter Four: **NEUROIMAGING BIOMARKERS OF DEMENTIA IN PATIENTS WITH AMNESTIC *VS.* NON-AMNESTIC MCI**

4.1 Introduction

Mild cognitive impairment (MCI) is a transitional stage between healthy aging and dementia. Each year, 5-15% of MCI patients convert from MCI to dementia (Petersen *et al*., 1999). The rate of conversion to clinical AD and dementia are lower for epidemiological studies compared to clinical studies (Ritchie et al., 2001, Larrieu et al., 2002). Currently there are 16 different definitions of MCI in the literature (Matthews *et al*. 2008), but they all include a core set of criteria. MCI is defined as having self- or informant-reported cognitive complaints with evidence of objective cognitive impairment, but essentially preserved activities of daily living.

The concept of MCI was introduced in the 1980s by Reisberg and colleagues, further conceptualized by Petersen *et al*. (1999) and revised under Winblad *et al*. (2004) to include clinical subtypes. These subtypes are defined according to the presence or absence of episodic memory impairment (amnestic or non-amnestic) and number of cognitive domains affected (single domain or multiple domain). It has been shown that progression to AD is more closely related to certain subtypes, such that being amnestic and multiple-domain MCI both increases the likelihood of progression (Hunderfund et al., 2006, Fischer et al., 2007, Forlenza et al., 2009). However, not all patients progress onto dementia, as many as 40% to 70% of all MCI patients revert to normal cognition or remain stable at follow-up (Ganguli *et al*., 2010).

MCI consists of both a clinically and etiologically heterogeneous patient population. Typically, pathological changes observed in MCI resemble earlier versions of those observed in dementia. A recent study from the Mayo Clinic Study of Aging highlighted the heterogeneous nature of MCI using biomarkers for β-amyloid and neuronal injury (Petersen *et al*., 2013). It was

found that 43% of MCI patients showed positive amyloid and neuronal injury biomarkers, 14% were amyloid positive only, 29% had neuronal injury biomarkers only, and 14% had neither amyloid nor neuronal injury biomarkers. Considering the inherent heterogeneity of clinical MCI, our study used three specific neuroimaging biomarkers—PiB-PET for β-amyloid, WMH for vascular injury and ASL MR for cerebral perfusion —to investigate the etiological and pathological differences between amnestic MCI (aMCI) and non-amnestic MCI (naMCI).

The amyloid cascade hypothesis, stating that β-amyloid deposition in the brain parenchyma precipitates downstream AD pathology, is one of the leading frameworks for AD research (Hardy and Higgins 1992). PiB-PET has previously been used to demonstrate differences in β-amyloid accumulation between cognitively healthy controls, MCI and AD (Devanand *et al*., 2010; Hatashita and Yamasaki 2013; Zhang *et al*., 2014). However, few studies have looked at the differences between MCI subtypes. β-amyloid accumulation has been commonly reported in amnestic MCI, but studies of β-amyloid accumulation in naMCI have not been as consistent, with some groups suggesting β-amyloid levels within the healthy aging range (Pike *et al*., 2007) and others suggesting β-amyloid levels similar to that of aMCI (Wolk *et al*., 2009).

The vascular contributions to cognitive decline have also been explored in literature (Gorelick *et al*., 2011); however, studies examining the association of WMH to MCI subtypes have been inconclusive to date. There have been reports of higher WMH in aMCI (Luchsinger *et al*., 2009), naMCI (Mariani and Mecocci 2007) or no association with either MCI subtype (He *et al*., 2009, van de Pol *et al*., 2009) compared to controls. Thus the purpose of our study is to investigate biomarker surrogates of pathological changes in MCI subtypes, which might underlie

future conversion to dementia, accounting for both β-amyloid accumulation and vascular pathology related to white matter lesions.

β-amyloid accumulation occurs early in the Alzheimer's disease process (Rinne *et al*., 2010, Villemagne *et al*., 2011), with subsequent neuronal cell death leading to decreased glucose metabolism (Engler *et al*., 2006) and progression to cortical atrophy (Koivunen *et al*., 2011). According to the NIA-AA criteria, FDG-PET-measured hypometabolism reflects downstream neuronal injury that is associated with AD. Since FDG-metabolism has been shown to tightly couple with cerebral perfusion, we can apply these findings to our study (Raichle, 1998). Jauhiainen *et al*. (2008) showed that both aMCI and naMCI exhibit hypometabolism in the posterior cingulate compared to control. aMCI also exhibited a characteristic medial temporal lobe hypometabolism compared to naMCI and controls. Depending on the study, there are a number of different regional hypometabolism reported. Nishi *et al*. (2010) also found hypometabolism in the right medial temporal cortex, right prefrontal cortex, left superior parietal cortex, and bilateral posterior cingulate cortex in MCI patients with impaired delayed recall in memory. In MCI with executive function impairment, hypometabolism was detected in the right prefrontal cortex only. Reports have also found that hypometabolism in certain regions of MCI, such as posterior cingulate cortex can predict conversion to AD within 1-3 years (Chen *et al*., 2011, Shaffer *et al*., 2013, Caroli *et al*., 2015).

In this chapter, mean cortical PiB, WMH volume and regional cerebral perfusion are compared in patients with amnestic *vs*. non-amnestic MCI. We hypothesized that amnestic MCI patients will have higher mean cortical PiB retention, lower WMH burden and reduced perfusion in regions affected by AD pathology (including temporal cortex, angular gyrus and posterior cingulate gyrus). In non-amnestic MCI patients, we hypothesized that there will be lower mean

cortical PiB retention, higher WMH burden and reduced perfusion in the frontal pole and anterior cingulate cortex. However, due to the small sample size (n = 18), we regard this study as primarily exploratory and hypothesis generating.

4.2 Results

4.2.1 Characterization of Amnestic and Non-amnestic MCI

Cohort inclusion and exclusion criteria, and the methods for categorizing MCI patients as aMCI *vs*. naMCI, are provided in Chapter 2. In total, 18 patients with MCI were analyzed. Twelve patients were classified as aMCI, according to evidence of episodic memory impairment shown on neuropsychological testing, with or without additional impairments in other domains. There were 9 single domain aMCI and 3 multiple-domain aMCI. However, because of the small sample size we did not further analyze multiple domain *vs*. single domain amnestic MCI, but rather analyzed them as a single group. Six of eighteen MCI patients were classified as naMCI, according to impairment with executive function and/or processing speed. There was an even split of single and multiple-domain within the naMCI cohort. Consistent with previous literature, there was a higher prevalence of aMCI compared to naMCI within our study cohort (Yaffe *et al*., 2006, Forlenza *et al*., 2009).

The neuropsychological profile revealed significant differences between MCI subtypes in agreement with their assigned categorization. As shown in Table 4-1, aMCI scored lower on episodic memory (z = -2.43) compared to naMCI (z = -0.15), $p < 0.0001$. naMCI scored lower on both processing speed (z = -1.18; $p = 0.02$) and executive function (z = -1.91; $p = 0.05$) compared to aMCI. The MMSE scores did not differ significantly between aMCI (27.8 ± 1.64) and naMCI (28.3 ± 1.37), $p = 0.53$.

Between the MCI subtypes, there were also no significant differences with respect to age (aMCI: 74.2 ± 8.04 years old; naMCI: 74.0 ± 10.3 years old, $p = 0.97$), education (aMCI: 15.7 ± 2.10 years; naMCI: 14.2 ± 2.86 years, $p = 0.22$) and sex (aMCI: 33% female; naMCI: 83% female, $p = 0.13$). Based on the clinical profile described in Table 4-1, we did not detect any significant differences between the variables that could serve as potential confounders to our analyses of neuroimaging parameters.

Table 4-1 Subject characteristics of amnestic and non-amnestic MCI

Variable	**Amnestic MCI** n = 12	**Non-amnestic MCI** n = 6	***p*-value**
Age (years ± SD)	74.2 ± 8.04	74.0 ± 10.3	0.97
Sex (% female)	33	83	0.13
Education (years ± SD)	15.7 ± 2.10	14.2 ± 2.86	0.22
MMSE (±SD)	27.8 ± 1.64	28.3 ± 1.37	0.53
Clinical Profile (n)			
Current smoker	1 (8.3%)	0 (0%)	1.00
Diabetes	2 (17%)	1 (17%)	1.00
Hypertension	12(100%)	6 (100%)	1.00
Hypercholesterolemia	5 (42%)	5 (83%)	0.15
Depression (within 2 years)	5 (42%)	3 (50%)	1.00
Depression (prior diagnosis)	6 (50%)	3 (50%)	1.00
Lacunar infarcts	1 (8.3%)	1 (17%)	1.00
Lobar CMB	1 (8.3%)	0 (0%)	1.00
Deep CMB	1 (8.3%)	1 (17%)	1.00
Neuropsychological Profile (z score)			
Executive function	**-0.66**	**-1.91**	**0.02**
Episodic memory	**-2.43**	**-0.15**	**<0.0001**
Processing speed	**-0.23**	**-1.18**	**0.05**

Key – MMSE = Mini-Mental Status Examination, CMB = cerebral microbleeds

4.2.2 Neuroimaging Biomarker Characteristics

Methods for measurement of mean cortical PiB, WMH and regional and grey matter cerebral perfusion are described in Chapter 2. As shown in Table 4-2, there were no significant differences in PiB between aMCI (1.32 ± 0.08) and naMCI (1.25 ± 0.07), $p = 0.57$. Although not significant, applying the DVR cut-off of 1.25, 42% of aMCI were PiB+ and 50% of the naMCI were PiB+, $p = 1.00$.

The WMH burden, analyzed as a percentage of intracranial volume to account for head size (% WMH), was comparable between the two subtypes (aMCI: 0.11%, IQR 0.04% to 0.50%; naMCI: 0.17%, IQR 0.04% to 0.41%, $p = 0.94$). Logarithmic transformation was also applied to % WMH to account for the right-skewed distribution (aMCI: -1.00, IQR -1.38 to -0.32; naMCI: -0.81, IQR -1.48 to -0.43), $p = 0.44$.

Cerebral perfusion differences between aMCI and naMCI were found in the left temporal gyrus and anterior cingulate cortex (Figure 4-1). There was lower cerebral perfusion in the left temporal gyrus of aMCI (40.1 ± 11.7 mL/100g/min) compared to naMCI (53.1 ± 12.3 mL/100g/min), $p = 0.04$. Contrary to our hypothesis, there was also lower cerebral perfusion in the anterior cingulate cortex of aMCI (42.9 ± 4.7 mL/100g/min) compared to naMCI (49.0 ± 7.2 mL/100g/min), $p = 0.04$. There were no significant differences in the perfusion of grey matter, AD-ROI, left and right angular gyrus, right temporal gyrus and frontal pole between aMCI and naMCI.

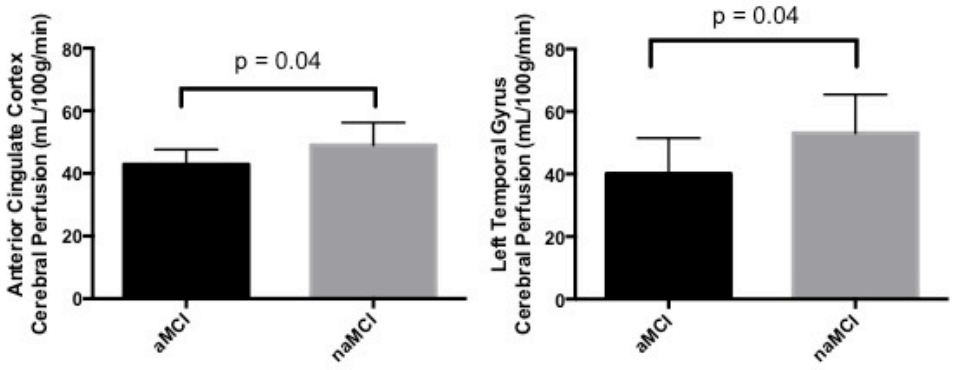

Figure 4-1 Comparing amnestic (n = 12) to non-amnestic (n = 6) MCI patients, there were significantly lower cerebral perfusion in the anterior cingulate cortex (aMCI: 42.9 ± 4.7 mL/100g/min, naMCI: 49.0 ± 7.2 mL/100g/min, $p = 0.04$) and left temporal gyrus (aMCI: 40.1 ± 11.7 mL/100g/min; naMCI: 53.0 ± 12.3 mL/100g/min, $p = 0.04$).

Table 4-2 Neuroimaging biomarker characteristics of amnestic and non-amnestic MCI

Variable	**Amnestic MCI** n = 12	**Non-amnestic MCI** n = 6	***p*-value**
PiB DVR (±SD)	1.32 ± 0.29	1.25 ± 0.16	0.57
PiB+ (DVR >1.25, %)	5 (42%)	3 (50%)	1.00
% WMH (IQR)	0.11 (0.04 to 0.50)	0.17 (0.04 to 0.41)	0.94
Log % WMH (IQR)	-1.00 (-1.38 to -0.32)	-0.81 (-1.48 to -0.43)	0.44
Arterial Spin Labeling Perfusion (mL/100g/min, ±SD)			
Grey Matter	45.2 ± 4.7	49.0 ± 3.9	0.11
AD-ROI	50.0 ± 7.3	50.9 ± 5.8	0.80
L Angular Gyrus	49.4 ± 10.4	50.3 ± 8.2	0.86
R Angular Gyrus	48.0 ± 5.8	50.9 ± 5.0	0.32
L Temporal Gyrus	**40.1 ± 11.7**	**53.1 ± 12.3**	**0.04**
R Temporal Gyrus	44.0 ± 11.5	47.5 ± 6.7	0.50
Posterior Cingulate Cortex	55.7 ± 10.5	51.9 ± 12.1	0.50
Anterior Cingulate Cortex	**42.9 ± 4.7**	**49.0 ± 7.2**	**0.04**
Frontal Pole	47.3 ± 7.4	52.5 ± 7.2	0.17

Key - WMH = white matter hyperintensity, IQR = interquartile range, ROI = region of interest, L = left, R = right

4.3 Discussion

Our exploratory study is among the first to investigate three neuroimaging biomarkers that highlight different pathological processes within the same MCI cohort in order to characterize subtypes. Contrary to what was hypothesized, we did not find a significant difference between MCI subtypes in PiB retention and WMH burden, two biomarkers chosen to represent the two most common aetiologies that lead to dementia. However, we did find a significantly lower cerebral perfusion in two pre-specified regions (left temporal gyrus and ACC) in the amnestic MCI compared to non-amnestic MCI. When considering all of these findings together, we may be able to provide some insight on MCI subtypes.

Many studies have analyzed β-amyloid levels in MCI subtypes (Pike *et al*., 2007; Wolk *et al*., 2009). The consensus from these studies suggests that aMCI have the highest proportion of β-amyloid retention, and even greater if it involves impairment in additional cognitive domains. On the other hand, there is controversy regarding whether naMCI is associated with β-amyloid retention. In our study, naMCI had a PiB DVR of 1.23 ± 0.29, which is still a considerable level of β-amyloid accumulation. One proposed explanation for amyloid-positive naMCI is that it may be an atypical phenotype of early AD (Wolk *et al*., 2009), while amyloid negative naMCI may be caused by non-AD pathologies.

Recent evidence suggests that neuronal injury and thus neurodegeneration could emerge from non-amyloid pathway even in regions that are typically affected in AD (Wirth *et al*., 2013). "Suspected non-AD pathology" (SNAP) have been studied in the cognitively normal elderly population, where neuroimaging biomarkers of brain injury were found in the absence of amyloidosis (Knopman *et al*., 2012). The term SNAP-MCI was recently introduced to describe the phenomenon in the MCI population (Petersen *et al*., 2013; Caroli *et al*., 2015). SNAP could

explain why we saw differences in cerebral perfusion between aMCI and naMCI despite similar amyloid levels. However, based on our study WMH is not the SNAP that underlies the perfusion differences, because WMH volumes were similar between aMCI and naMCI.

The lack of significant difference between WMH volume in aMCI and naMCI was contrary to our hypothesis. We hypothesized that naMCI, encompassing executive dysfunction, would be attributed primarily to vascular pathology. The basis of this hypothesis was that WMH impairs executive function due to subcortical-frontal connectivity (Vermeer *et al*., 2013); therefore, we reasoned that WMH pathology should be more prevalent in naMCI. Although this hypothesis is rational, it may oversimplify the role of WMH in naMCI and executive function. The Leukoaraiosis And DISability (LADIS) study showed that WMH are associated with worse performance on immediate and delayed memory, processing speed and executive function (Au *et al*., 2006; Debette & Markus 2010). Considering the comparable levels of WMH in our aMCI and naMCI cohort, it may be that the role of WMH extends beyond executive function impairment in naMCI.

A limitation for interpreting our WMH findings is that our study did not account for the effects of APOE ε4. There is evidence to suggest the interactive effect of WMH and APOE ε4 with respect to frontal executive functions, such that cognitive performance is worse in APOE ε4 carriers compared to non-carriers (Yoon *et al*., 2013). Thus, we cannot eliminate the possibility of APOE ε4 as a confounder.

The most intriguing aspect of this preliminary study is the significant ASL-MR perfusion finding in two regions, left temporal gyrus and anterior cingulate gyrus, despite non-significant findings for PiB retention and WMH burden. Most of the studies to date examine how MCI and

subtypes differ compared to AD or controls, but differences between subtypes can also highlight the underpinning of MCI.

Parietal and temporal hypometabolism are characteristic features that distinguish AD from controls (Silverman *et al*., 2001), and often precedes structural atrophy in brain regions susceptible to AD pathology (Chetelat *et al*., 2008). FDG-PET studies also show that hypometabolism is found in the parietotemporal and posterior cingulate cortex of MCI patients and may characterize future converters to AD (Chetelat *et al*., 2003; Drzezga *et al*., 2003; Mosconi *et al*., 2008). Comparing between MCI subtypes, Jauhiainen *et al*. (2008) showed hypometabolism in the medial temporal lobe of aMCI compared to the naMCI and controls. Consistent with our finding that aMCI patients have lower perfusion in the left temporal gyrus, these studies also demonstrate that the temporal gyri are regions susceptible to perfusion and metabolic changes of AD pathology.

Chao *et al*. (2009) was among the first to compare ASL perfusion in 12 single domain aMCI and 12 single-domain naMCI patients compared to healthy controls. They found that aMCI patients exhibited hypoperfusion in the bilateral posterior cingulate relative to controls, which is consistent with previous SPECT and PET studies (Johnson *et al*., 2005; Caroli *et al*., 2007). naMCI with executive function impairment exhibited hypoperfusion in the middle frontal cortex, bilateral posterior cingulate and left precuneus compared to the controls. Comparing between MCI subtypes, they found hypoperfusion in the left posterior cingulate cortex of naMCI compared to aMCI, which our study did not observe.

Chao and colleagues (2009) also showed additional regions of hypoperfusion in the middle frontal cortex and left precuneus of naMCI compared to aMCI patients, which may be

worthwhile adding into future analyses. Our lack of replication of these findings may be related to differences between the study cohorts in the causes of MCI, or a possible type II error.

Other groups have studied variants of MCI subtypes that we are interested in (Seo *et al*., 2008). Seo *et al*. compared metabolism, measured by FDG-PET, in amnestic MCI (expected prodromal AD) and subcortical vascular MCI (expected prodromal SVaD). They found that aMCI showed hypometabolism in the medial temporal region and orbitofrontal cortex (OFC). The observed hypometabolism in the medial temporal region was consistent with expectations, since hypometabolism is likely attributing to the memory deficits in aMCI. However, they also found hypometabolism in the OFC of aMCI as opposed to svMCI, which was more difficult to explain. Likewise, we did not expect there to be hypoperfusion in the ACC of aMCI compared to naMCI. The reason for this paradox remains unclear, but it is possible that the decision making network is impacted in aMCI considering the functional connectivity between ACC and OFC (Cohen *et al*., 2005). In future studies, it may be worthwhile to consider investigating perfusion in more specific regions of the frontal lobe, including prefrontal cortex and OFC.

4.3.1 Study Limitations

The study could be improved by increasing the sample size to increase power and by doing so, it would permit for more in-depth analysis based on single- and multiple-domain classifications. We can gain more insight when we make this type of categorization since amnestic multiple-domain MCI is more likely to progress to AD-dementia (Wolk *et al*., 2009). We also did not have APOE ε4 genotyping (Drzezga *et al*., 2005), which limited our understanding of this MCI cohort, and the extent to which other factors aside from neuropathology could be attributing to the cognitive impairment and classification. Lastly, without a control group, conclusions drawn

were relative between the two MCI subtypes, and we could not comment on the direction of change relative to a healthy aging group based on our data alone.

4.3.2 Conclusions and Future Directions

We regard this study as preliminary and primarily hypothesis generating, though there are some key findings that merit discussion. We found differences in regional perfusion between MCI subtypes, such that hypoperfusion was found in the left temporal gyrus and ACC of aMCI compared to naMCI. These findings were found in the absence of differences in β-amyloid accumulation and white matter lesions, as evidenced on neuroimaging, between the MCI subtypes. Based on the findings, we would further investigate into the involvement of other neuropathology beyond amyloid and vascular contributions. The amyloid cascade hypothesis has been criticized as being too simplistic (Castello *et al*., 2014). Thus it may be worthwhile investigating the other contributors of AD such as tau (Maccioni *et al*., 2010) in MCI subtypes. PET tau radiotracers have been developed to map neurofibrillary tangles *in vivo* (Shah *et al*., 2014), and should be considered for future experiments.

Future studies could also focus on the role of prefrontal networks in aMCI and naMCI, since our positive finding in ACC show that there may be detectable, and thus potentially important, changes. It has been shown that frontal hypometabolism is frequently detected in multiple-domain MCI patients (Mosconi *et al*., 2008) and with a larger sample size, this difference may be further elucidated. We can also investigate the relationship between regional WMH, such as periventricular and subcortical, and regional cerebral perfusion in the MCI subtypes.

With the use of a control group, it would also be worthwhile investigating differences in perfusion between each MCI subtype and healthy controls. In the absence of a control group, we cannot determine whether perfusion might be pathologically increased or decreased in aMCI *vs*. naMCI but to a similar degree. For example, if there are compensation mechanisms for MCI, with consequent hyperperfusion in the metabolically active compensating regions, we would only be able to detect this in our study if the compensatory regions differed in aMCI and naMCI.

By having a better understanding of MCI subtypes, it may be possible to better detect the underlying pathologies of MCI, providing better prognosis and potentially allowing for future specific prevention strategies targeted against specific pathological subtypes.

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Chapter Five: **RELATIONSHIP BETWEEN B-AMYLOID AND WHITE MATTER LESIONS WITH CEREBRAL PERFUSION IN THE EARLY STAGES OF COGNITIVE DECLINE**

5.1 Introduction

Alzheimer's disease (AD) and vascular dementia (VaD) are the two most common causes of dementia. AD is a neurodegenerative disorder characterized by accumulation of β-amyloid and neurofibrillary tangles. VaD results from cerebrovascular disease, such as macrovascular (*i.e.*, subcortical and cortical infarcts) or microvascular (*i.e.*, white matter lesion) insults that lead to cognitive impairment. Concurrent AD and VaD etiology may lead to synergistic effect on cognitive decline and this combination is diagnosed as mixed dementia (Attems and Jellinger, O'Brien and Markus, 2014). Neuroimaging techniques such as FDG-PET, structural MR imaging, SPECT and ASL-MR imaging have been used extensively in the literature to explore key pathomechanisms of dementia, including alterations to cerebral metabolism, blood flow and structural atrophy.

One of the existing debates in literature is to understand the cause and effect of cerebral hypoperfusion on cognitive decline. It has been argued that cerebral hypoperfusion may reflect neuronal injury, which is a consequence of pathomechanisms underlying dementia; however others argue that hypoperfusion, independent of other pathomechanisms, directly causes cognitive deficits (Austin *et al*., 2011). Patterns of cerebral hypoperfusion are commonly reported in MCI and AD literature (Johnson *et al*., 2005), and sometimes occur in the same region with β-amyloid accumulation (Mattsson *et al*., 2014) or small vessel disease (Brickman *et al*., 2009). It has been proposed that cerebrovascular disease, including small vessel disease, may interact with AD pathology and inhibit clearance of β-amyloid, thus promoting further deposition (Zlokovic, 2011). However, many studies to date have shown that there is a poor

correlation between vascular disease and amyloid load, suggesting that the two underlying processes are likely independent predictors of cognitive decline (Hedden *et al*., 2012, Gurol *et al*., 2013, Vemuri *et al*., 2015).

The relationship between β-amyloid accumulation and cerebral metabolism and perfusion has been studied to some extent in literature, using both FDG-PET and ASL MR. Forster *et al*. (2012) showed that the pattern of β-amyloid accumulation precedes the pattern of hypometabolism anatomically, in regions such as the bilateral frontal, lateral temporal, retrosplenial and parietal cortex, though with a temporal delay. This finding support the theory that neuronal dysfunction is subsequent to β-amyloid accumulation. Using a dual PiB and FDG-PET tracer, Fu *et al*. (2014) showed increased β-amyloid accumulation and hypometabolism in the parietotemporal areas, PCC/precuneus and medial temporal cortex. These biomarker changes become gradually worse with disease progression from MCI to AD. Similar findings were elucidated where higher β-amyloid was related to lower ASL perfusion in the temporo-parietal regions, regardless of control, MCI or AD (Mattsson *et al*., 2014).

The association between WMH burden and decreased cerebral perfusion in AD has not been clearly established in literature. Zhang *et al*. (2012) described the lack of association between WMH burden and ASL perfusion in a composite ROI (Landau *et al*., 2011) susceptible to AD pathology. On the contrary, Li and Hu (2014) found decreased FDG-PET metabolism in the grey matter, frontal and parietal lobes in the presence of white matter ischemic lesions. Kimura *et al*. (2012) also showed, using SPECT imaging, that decreased perfusion in the anterior cingulate gyrus and insula were associated with white matter lesions in AD patients, compared to those without these lesions. These observations suggest that the pattern of hypoperfusion seen with AD pathology and WMH may be distinct.

Most of the studies in the literature focus on studying the relationship of either β-amyloid or vascular pathology with cerebral hypoperfusion in patients with dementia, but few studies have focused on examining the MCI or SCC population. Therefore, we investigated the relationship of both β-amyloid accumulation and WMH burden with grey matter and regional cerebral perfusion in a group of patients with CDR 0.5, previously classified as SCC or MCI depending on their neuropsychological performance. We hypothesized that regional cerebral perfusion will be inversely correlated with both β-amyloid accumulation and WMH burdens in pre-specified ROIs commonly impacted in AD and VaD. More specifically, we expect to see an inverse relationship between β-amyloid accumulation and cerebral perfusion in ROIs affected in AD, and an inverse relationship between WMH burden and cerebral perfusion in ROIs affected in VaD. If our hypothesis is confirmed, our findings would therefore demonstrate that hypoperfusion, potentially reflecting neuronal injury, would be a detectable consequence of β-amyloid and vascular changes even in patients in the earliest stages of neurodegeneration.

5.2 Results

5.2.1 Characterization of Study Population

Cohort inclusion and exclusion criteria are provided in Chapter 2. Characteristics of the study cohort are shown in Table 5-1. The mean age was 74.8 ± 7.3 years old, 47.5% were female, and with an average level of education of 16.3 years. Mean cortical PiB retention was 1.24 ± 0.22 and 31.1% of patients exceeded the PiB cut-off value of 1.25. The median value of WMH, expressed as a percent of intracranial volume was 0.22 (IQR 0.10 to 0.56). When this value was logarithmically transformed to better fit a normalized distribution, the median log percentage WMH was -0.64 (IQR -0.99 to -0.25).

Table 5-1 Subject characteristics of patients with early cognitive decline (N = 61)

Variable	
Age (years±SD)	74.8 ± 7.3
Sex (% female)	47.5
Education (years±SD)	16.3 ± 2.5
MMSE (mean±SD)	28.6 ± 1.3
Clinical Profile	
Current Smoker	6 (9.8%)
Diabetes	10 (16.4%)
Hypercholesterolemia	42 (68.8%)
Depression (within 2 years)	24 (39.3%)
Depression (prior diagnosis)	21 (34.4%)
Silent brain infarcts	9 (14.8%)
Lobar CMB	12 (19.7%)
Deep CMB	3 (4.9%)
PiB+ (DVR ≥1.25)	19 (31.1%)
% WMH (IQR)	0.22 (0.10 to 0.56)

5.2.2 Relationship between ASL Perfusion and β-Amyloid Retention

Figure 5-1 shows the significant negative correlation between β-amyloid retention and cerebral perfusion in the left temporal gyrus ($r = -0.31, p = 0.02$) and right temporal gyrus ($r = -0.25, p = 0.05$). This relationship was not observed in the other prespecified regions (grey matter, left and right angular gyrus, posterior cingulate cortex, AD-ROI, ACC and frontal pole, Table 5-2). We did not detect any significant relationships between age and β-amyloid retention in our study population ($r = 0.18, p = 0.15$). However, we also performed multivariable analyses adjusting for age based on known correlations between age, β-amyloid accumulation and lower cerebral perfusion in normative studies (Table 5-3), and found that β-amyloid accumulation was inversely correlated to perfusion only in the right temporal gyrus. With a sample size of 61 patients, we had 80% power to detect correlations with $r = 0.31$ or greater.

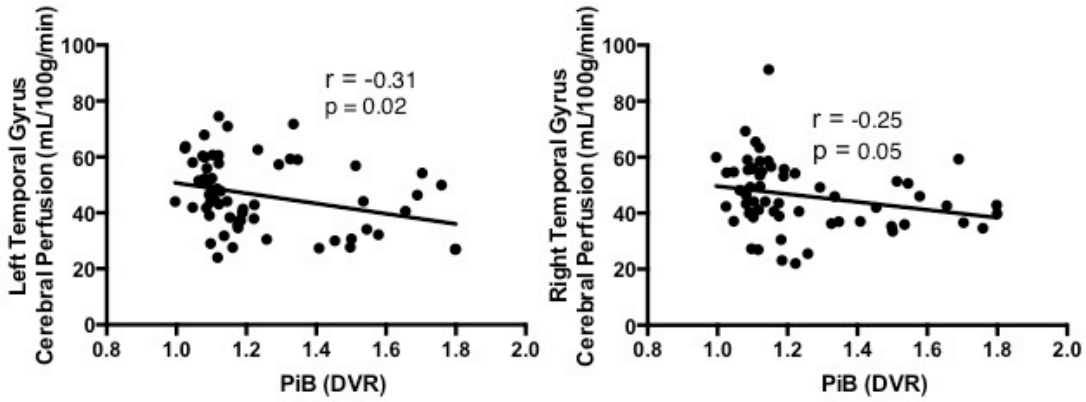

Figure 5-1 The inverse relationship between mean cortical PiB (DVR) and left (r = -0.31, p = 0.02) and right (r = -0.25, p = 0.05) temporal gyrus perfusion (mL/100g/min) in patients with early cognitive decline (N = 61).

Table 5-2 Pearson's correlation of PiB DVR and regional ASL perfusion in patients with early cognitive decline (N = 61)

	Correlation Coefficient	***p*-value**
Grey Matter	r = -0.16,	0.23
AD-ROI	r = -0.18	0.18
Left Angular Gyrus	r = -0.03	0.79
Right Angular Gyrus	r = -0.08	0.52
Left Temporal Gyrus	***r* = -0.31**	**0.02**
Right Temporal Gyrus	***r* = -0.25**	**0.05**
Posterior Cingulate Cortex	r = -0.06	0.64
Anterior Cingulate Cortex	r = -0.06	0.66
Frontal Pole	r = -0.09	0.51

Table 5-3 Multivariable-adjusted analysis (controlling for age) of PiB DVR and regional ASL perfusion in patients with early cognitive decline (N = 61)

	***β*-Coefficient**	**95% CI**	***p*-value**
Grey Matter	-3.37	-10.5 to 3.71	0.35
AD-ROI	-6.02	-17.1 to 5.03	0.28
Left Angular Gyrus	-5.79	-17.9 to 6.30	0.34
Right Angular Gyrus	-1.96	-14.0 to 10.1	0.75
Left Temporal Gyrus	**-17.3**	**-32.2 to -2.27**	**0.02**
Right Temporal Gyrus	-13.4	-27.8 to 1.02	0.07
Posterior Cingulate Cortex	-7.95	-24.8 to 8.91	0.35
Anterior Cingulate Cortex	-1.51	-10.2 to 7.16	0.73
Frontal Pole	-2.62	-12.2 to 6.97	0.59

Key – CI = confidence interval

5.2.3 Relationship between ASL Perfusion and White Matter Hyperintensity Burden

We found a significant negative correlation between ASL perfusion and log percentage WMH burden in the grey matter, right temporal gyrus and posterior cingulate cortex. As shown in Table 5-4, this relationship was not found in the AD-ROI, left and right angular gyrus, left temporal gyrus, nor in either of the ROIs, ACC and frontal pole, thought to be impacted by WMH burden in vascular dementia.

Table 5-4 Pearson's correlation of log percentage WMH and regional ASL perfusion in patients with early cognitive decline (N = 61)

	Correlation coefficient	***p*-value**
Grey Matter	***r* = -0.33**	**0.01**
AD-ROI	*r* = -0.24	0.06
Left Angular Gyrus	*r* = -0.19	0.14
Right Angular Gyrus	*r* = -0.16	0.23
Left Temporal Gyrus	*r* = 0.10	0.45
Right Temporal Gyrus	***r* = -0.34**	**0.01**
Posterior Cingulate Cortex	***r* = -0.30**	**0.02**
Anterior Cingulate Cortex	*r* = -0.16	0.21
Frontal Pole	*r* = -0.22	0.09

We did find a significant relationship between age and WMH burden ($r = 0.48, p < 0.001$). After multivariable-adjusted analysis controlling for age (Table 5-5), decreased ASL perfusion was still found in the grey matter ($\beta = -1.45, p = 0.04$) and right temporal gyrus ($\beta = -3.26, p = 0.01$) (Figure 5-2).

Table 5-5 Multivariable-adjusted analysis (controlling for age) of log percentage WMH and regional ASL perfusion in patients with early cognitive decline (N = 61)

	***β*-Coefficient**	**95% CI**	***p* value**
Grey Matter	**-1.45**	**-2.79 to -0.10**	**0.04**
AD-ROI	-1.37	-3.53 to 0.79	0.21
Left Angular Gyrus	-1.28	-3.65 to 1.08	0.28
Right Angular Gyrus	-0.45	-2.81 to 1.91	0.70
Left Temporal Gyrus	2.29	-0.72 to 5.31	0.13
Right Temporal Gyrus	**-3.62**	**-6.37 to -0.86**	**0.01**
Posterior Cingulate Cortex	-3.12	-6.35 to 0.11	0.06
Anterior Cingulate Cortex	-0.99	-2.67 to 0.69	0.24
Frontal Pole	-1.45	-3.30 to 0.39	0.12

Key - CI = confidence interval

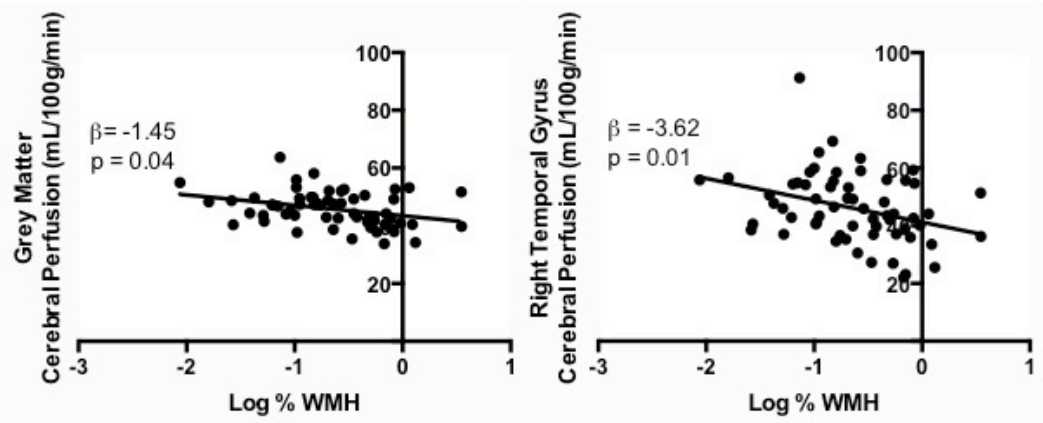

Figure 5-2 Age-adjusted relationship between log % WMH and cerebral perfusion in the grey matter and left temporal gyrus in patients with early stage of cognitive decline (N = 61).

5.3 Discussion

This was the first study to use ASL, a novel technique for measuring cerebral perfusion, to simultaneously show the relationship between cortical and regional cerebral perfusion with β-amyloid retention and WMH burden in a population with CDR 0.5, indicating memory concerns. The inclusion criteria of the study required participants to have hypertension, in order to enrich the cohort for small vessel disease. Mean cortical PiB value was 1.24 showing substantial β-amyloid deposition that is not far from DVR cut-off of 1.25 often used to label PiB positivity. Based on these two characteristics of the cohort, the study design was suitable for investigating the relationship between both biomarkers of dementia neuropathology and regional cerebral perfusion.

We revealed an inverse relationship between both β-amyloid accumulation and white matter lesion volume with cerebral perfusion in select regions of the brain susceptible to AD, but not VaD. Hypoperfusion in the right temporal gyrus was correlated with both increasing levels of PiB retention and WMH burden. Within the left temporal gyrus, there was an inverse

relationship between PiB retention and cerebral perfusion. Hypoperfusion in the cortical grey matter and posterior cingulate was correlated with increasing levels of WMH burden.

The relationship between increased PiB retention and decreased perfusion in the left and right temporal gyrus were consistent with previous literature findings (Forster *et al*., 2012; Fu *et al*., 2014). Using the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, Mattsson *et al*., (2014) found increasing β-amyloid retention in the presence of lower cerebral perfusion in the temporo-parietal regions regardless of the disease stage. Interestingly, a positive correlation was also detected between β-amyloid accumulation and perfusion in the posterior cingulate cortex across each of the disease stage (control, MCI and AD). Our study also examined these ROIs including angular gyrus of the parietal region and posterior cingulate cortex, but did not detect significant relationships between β-amyloid deposition and cerebral perfusion. However, our study differed by focusing on a population with MCI and SCC–in the study from ADNI, SCC would have been considered part of the control group.

Since cerebral glucose metabolism and perfusion are coupled (Raichle 1983), we can also draw comparisons to literature using FDG-PET as a surrogate for perfusion. A population-based study found that cognitively healthy patients with PiB positivity had hypometabolism in regions frequently affected in AD and that higher levels of PiB predicted greater hypometabolism (Lowe *et al*., 2014), a finding consistent with our study. This same study also elucidated that APOE ε4 did not affect the degree of hypometabolism. However, Jagust and Landau (2012) reported that APOE ε4 status is directly linked to hypometabolism, irrespective of β-amyloid deposition. Future studies should examine the relationship between APOE ε4 and hypoperfusion, a measurement coupled to hypometabolism but not identical, in the presence of β-amyloid deposition.

Although our study did not detect hyperperfusion in the presence of β-amyloid deposition, a growing collection of literature has focused on describing this relationship. The hippocampus and medial temporal lobe were regions particularly susceptible to this effect, and these findings have been reported within the cognitive healthy population (Oh *et al*., 2014), cognitive healthy population at genetic risk of AD (Bangen *et al*., 2012) and MCI population (Cohen *et al*., 2009). The hyperperfusion is thought to reflect a compensatory mechanism for β-amyloid neurotoxicity, or that β-amyloid accumulation drives increased neural activity requiring hyperperfusion (Ossenkoppele *et al*., 2013). It is difficult to pinpoint why the effect, especially in the medial temporal lobe, was not found in our study or consistently reported across literature. One explanation may be that compensatory hyperperfusion occurs only during a limited time period during disease progression, such that it disappears as pathology becomes more widespread and begins to affect the compensatory circuits themselves.

We found that WMH was associated with hypoperfusion in the cortical grey matter, right temporal gyrus and PCC. After adjusting for age-related effects on WMH burden, hypoperfusion was observed only in the cortical grey matter and right temporal gyrus. However, we failed to confirm our hypothesis that WMH would be associated with reduced perfusion in the frontal cortex, specifically the anterior cingulate cortex and frontal pole.

It was originally thought that white matter lesions might be responsible for deficits related to frontal executive function, as observed in vascular dementia. Tullberg *et al*. (2004) found that reduced frontal FDG-metabolism is present in patients with white matter lesions, regardless of the location of the lesion. Schuff *et al*. (2009) replicated this finding in subcortical vascular dementia, where subcortical white matter lesions were associated with reduced perfusion in the frontal cortex. The relationship between small vessel disease and ASL perfusion,

while accounting for neurodegeneration, was first studied by Benedictus and colleagues (2014). They found that smaller normalized brain volumes and larger white matter lesion volumes were both independently associated with lower global and cortical perfusion in AD patients. However, the same association was not found in the control population, which consisted of a group of patients with subjective cognitive concerns. Some other studies also failed to detect significant correlations between white matter lesions and cortical glucose metabolism or blood flow in subcortical VaD (Sabri *et al*., 1999; O'Sullivan *et al*., 2002).

Negative correlation between white matter lesion volumes and regional perfusion has not been the only trend reported. Kraut *et al*. (2008) found that patients with stable or progressive WMH burden over the course of 8 years exhibited a different pattern of cerebral perfusion in response to white matter severity. Patients with progressive WMH accumulation had greater longitudinal increased perfusion in the right inferior temporal/fusiform gyrus, right anterior cingulate and left superior temporal gyrus, but decreased perfusion over time in the rostral left cingulate, right inferior temporal gyrus and right fusiform gyrus. The finding that perfusion increased over time as WMH accumulated may appear counterintuitive to previous findings from cross-sectional studies showing that WMH was associated with lower perfusion and metabolism (DeCarli *et al*., 1995; Sultzer *et al*., 2002), but this observation could be due to compensatory mechanisms. In our study we did not find any brain regions where WMH was associated with higher perfusion; instead, we found that WMH were associated with lower right temporal gyrus perfusion and overall cortical gray matter perfusion.

We also observed decreased perfusion in the cortical grey matter with increasing WM severity. Vernooiji *et al*. (2008) found that greater white matter lesion volumes are correlated with lower cerebral blood flow in the carotids and basilar artery. This is to say that decreased

global circulating cerebral blood flow could lead to long-term ischemia that is contributing to the white matter lesions, since both subcortical and periventricular WM derive blood flow from penetrating small arteries overlying the cortex (Nonaka *et al*., 2003). More research is necessary to draw conclusions based on the cause-and-effect of this relationship between grey matter hypoperfusion and white matter lesions.

5.3.1 Study Limitations

The main focus of our study was to examine the relationships between β-amyloid accumulation and white matter lesions with regional cerebral perfusion in earlier stages of cognitive decline, and therefore we have limited ability to draw conclusions about cerebral perfusion reductions in more advanced disease stages. We also did not take into consideration the potential effect of APOE ε4, since a few studies have suggested the interaction of APOE ε4 and age on CBF (Wierenga *et al*. 2013), and β-amyloid deposition in healthy subjects (Mielke *et al*., 2012). It would have also been useful if we accounted for partial volume effects (PVE), which may affect cerebral perfusion measurements in the presence of neurodegeneration (Benedictus *et al*., 2014). However, PVE correction would be expected to be more important for advanced disease stage analyses where brain atrophy is more advanced.

5.3.2 Conclusions and Future Directions

An inverse relationship could be found between β-amyloid accumulation and white matter lesion with cerebral perfusion in regions commonly affected in AD, but not VaD. Previous studies have shown that vascular and amyloid pathologies are independent predictors of cognitive decline in

the healthy aging population (Vemuri *et al*., 2015). Since these independent biomarkers of neuropathology were both correlated with lower perfusion, CBF may be a common pathway that reflects disease burden, *i.e*., neuronal injury, in the cognitive decline population. However, based on our study we cannot determine the cause-effect relationship between altered cerebral perfusion in the presence of β-amyloid accumulation and white matter lesion.

Future directions should examine the longitudinal relationship between β-amyloid burden and white matter lesions with cerebral perfusion in order to better understand possible perfusion compensation patterns. Similarly, replication of these findings in cross-sectional studies of SCC, MCI and AD specific groups could highlight important differences in biomarker relationships. It would also be worthwhile to focus on the relationship between regional perfusion and specific WMH regions (periventricular *vs*. subcortical, left *vs*. right hemisphere).

5.4 References

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Chapter Six: **CONCLUSIONS**

6.1 Summary of Research

The focus of our study was to understand neuropathological changes that occur in early cognitive decline. Both PiB-PET and white matter hyperintensity MR imaging were used to determine the prevalence of Alzheimer's disease pathology and cerebrovascular disease pathology in this population. ASL-MR imaging was also used to determine changes in cortical grey matter perfusion and in regions including left and right angular gyrus, left and right temporal gyrus, posterior cingulate cortex, a composite AD-affected region, anterior cingulate cortex and frontal pole. Our study is the first to examine these three neuroimaging biomarkers simultaneously in the SCC and MCI population.

In Aim 1, we did not detect differences in mean cortical β-amyloid retention and ASL perfusion in all the aforementioned ROIs between our SCC and MCI cohorts. However, there was significantly higher WMH burden in SCC compared to MCI. We postulated that white matter lesions may mediate subjective cognitive concerns, but are not sufficient to cause MCI, which is likely mediated by other pathologies in addition. Finding comparable levels of these neuroimaging biomarkers in SCC as MCI suggests that SCC also arises from heterogenous causes, like MCI. Further research studies will be needed to understand contributing pathophysiological mechanisms of SCC.

In Aim 2, we compared the three putative neuroimaging biomarkers of dementia between amnestic and non-amnestic MCI. Due to the small sample size, it was regarded as preliminary and primarily hypothesis generating. We did not detect any significant differences between β-amyloid retention and white matter lesions between the two subtypes, which was surprising because these two biomarkers represent the two most common etiologies of dementia, and prior

research, although sometimes conflicting, suggested that non-amnestic MCI may be less likely caused by AD pathology and more likely caused by non-AD pathologies such as cerebrovascular disease. ASL perfusion was lower in the left temporal gyrus and ACC of amnestic MCI compared to non-amnestic MCI. Hypoperfusion in the left temporal gyrus is likely contributing to, or reflective of, the memory deficits in amnestic MCI, but the clinical relevance of the ACC hypoperfusion requires further investigation. Future experiments are required to replicate these findings prior to drawing more conclusions.

In Aim 3, we determined the relationship between β-amyloid accumulation and white matter lesions with cerebral perfusion in the ROIs susceptible to changes in Alzheimer's disease and cerebrovascular disease. Within our cognitive decline population with CDR 0.5, we found that there was an inverse relationship between higher β-amyloid retention and lower perfusion in the left and right temporal gyrus, consistent with previous literature (Fu *et al*., 2014). Additionally, within the same cohort, we also found an inverse relationship between higher white matter lesion burden and lower cerebral perfusion in the cortical grey matter and right temporal gyrus, after adjusting for age. Since both β-amyloid and white matter lesions were correlated with hypoperfusion in the right temporal gyrus, hypoperfusion may be a common pathway in this region that reflect disease burden in earlier stages of cognitive decline.

These findings provide a better understanding of the extent to which SCC and MCI, and subtypes of MCI, may result from the two most common pathologies of dementia. Hypoperfusion in regions such as the temporal gyrus is associated with common neuropathologies underlying dementia and thus ASL-MR could be a useful and important technique to better understand cognitive decline in future studies. We also conclude from our study that the temporal gyrus was a region particularly susceptible to changes in early cognitive

decline, especially in our amnestic MCI cohort. It would be interesting to replicate this finding in the SCC population, but it is likely that sub-categorization of this heterogeneous population will be needed in order to detect a difference.

6.2 Study Limitations

Most of our study limitations lie in the retrospective nature of the study, where the research questions were formulated around an existing parent study. The sample size of the parent study was based on having sufficient power to detect longitudinal cognitive changes in the overall cohort. Because we subcategorized the cohort into SCC and MCI subtypes, the retrospectively determined aims of this thesis had less statistical power, especially in Aim 2, which was regarded as a preliminary and hypothesis-generating study. Re-evaluation of patients who were recruited to the parent study based on CDR 0.5 revealed that most were in fact SCC, and not MCI. As such, we also lacked statistical power to conduct single- and multiple-domain MCI comparison analyses. Therefore, the findings of this study warrant validation in a larger sample, multi-centered study.

Second, APOE ε4 genotyping was not included at the inauguration of the parent study in 2008. Thus, we could not account for the potential effects of APOE ε4 on age, or any of the neuroimaging biomarkers investigated (Caselli *et al*., 2009).

Third, by design, all the study participants had hypertension, and our findings may not be generalizable to non-hypertensive persons. However, hypertension is very common in the age ranges included in our study, being present in the majority of persons over 70 years; therefore, our findings are still relevant to a large segment of the population. Future studies should include both hypertensive and non-hypertensive persons, in order to compare them.

There use of the ROIs from the meta-analysis conducted by Landau *et al*. (2011) may have been a limitation, because these ROIs were fairly small and may have made our ASL findings susceptible to noise. Future studies should create masks of anatomical regions, including left and right temporal gyrus, left and right angular gyrus and posterior cingulate cortex, which can be applied to investigate regional ASL perfusion.

Because we considered our analyses to be exploratory, and we had a modest sample size with limited power, we did not perform adjustments for comparisons across multiple ROIs. Future studies will be needed to confirm our findings of lower perfusion in the left temporal gyrus and ACC in amnestic MCI compared to non-amnestic MCI.

Finally, we did not have a cognitive healthy patient group to serve as control. Therefore, we could not make conclusions on the disease burden of SCC, MCI, and MCI subtypes compared to their cognitively healthy counterparts.

6.3 Future Directions

Research in the field of dementia is currently booming in anticipation of an increasing aging population. Our study set a framework for understanding key etiological differences between SCC and MCI, and between MCI subtypes. Future research within this field will be necessary in order for successes in developing clinical trials, which are pushing to target earlier stages of the disease in hopes of achieving maximum therapeutic benefit.

Future research studies should focus on replicating our results, preferably in a population-based study, using a large sample size to adequately power the experiment and address relationships between neuroimaging biomarkers and neuropsychological performance during the early stages of cognitive decline. These measurements should also be obtained in reference to a

cognitive healthy control group, to account for effects that are inherent to healthy aging, and irrespective of dementia pathology. The role of APOE ε4 should also be considered in early cognitive decline, since it contributes to cognitive decline (Christensen *et al*., 2008; Cosentino *et al*., 2008) and is known to interact with neuroimaging biomarkers of dementia (Liu *et al*., 2015).

Extending on Aim 1, future experiments should conduct a longitudinal assessment of the effect of β-amyloid accumulation, white matter lesions and regional cerebral perfusion patterns on neuropsychological test performance in SCC. These relationships could help us understand more about SCC etiology, progression and possible ways to sub-categorize this heterogeneous cohort.

Since we focused on cortical PiB retention, it would also be interesting to whether regional PiB are more sensitive measurements to highlight underlying pathology in Aims 1 to 3. Though *in vivo* techniques such as ^{11}C-PiB and ^{18}F-florbetaben PET imaging have allowed us to better quantify neuropathology without relying on postmortem analyses, β-amyloid PET imaging cannot be used as a standalone marker for processes of early cognitive decline. Several novel PET ligands have shown promise in detecting tau pathology *in vivo* (Okamura *et al*., 2013; Chien *et al*, 2014). Therefore future experiments could expand on Aims 1 and 2 to investigate other non-amyloid and non-vascular causes of SCC and MCI.

The findings of our study can also extend beyond understanding etiology and contribute to other research for application purposes. In Aim 3, we highlight the relationship between neuropathology and decreased cerebral perfusion, therefore underlining the importance of preventing and/or treating modifiable vascular risk factors of cognitive decline. Previous studies have suggested the link between adequate perfusion and cognition (Binnewijzend *et al*., 2013). We can extend our objectives to investigate applications purposes of this study, such as how

exercise may improve global and regional ASL perfusion, and thus improve and/or prevent cognitive decline (Barnes *et al*., 2013). Since irreversible changes are not common during the earlier stages of dementia, targeted treatment to postpone or even prevent clinical onset of dementia during the early stages of cognitive decline is the most beneficial.

6.4 References

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