AMYLOID AND VASCULAR COGNITIVE IMPAIRMENT: A PILOT STUDY OF FREQUENCY AND IMPACT

by

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Abstract

Background

Traditionally, Alzheimer’s disease (AD) and vascular cognitive impairment (VCI) were considered to be distinct and unrelated; however, increasing evidence is demonstrating an overlap between AD and VCI pathology. As such, the current criteria for the clinical diagnosis of VCI may not distinguish those with cognitive impairment exclusively due to subcortical ischemic small vessel disease from those with mixed vascular and AD pathology. Furthermore, it is unclear how co-existing amyloid pathology may affect cognitive function in people with VCI. Objectives: 1) To determine the frequency of mixed VCI-amyloid pathology (mixed VCI) in patients clinically diagnosed with VCI. 2) To explore the impact of co-existing amyloid pathology on cognitive function in people with VCI.

Methods

This is a cross sectional analysis of data acquired from a randomized controlled trial investigating the effects of aerobic exercise training on cognitive function in people with VCI. Twenty-four participants – 18 participants with VCI and 6 normal controls – completed PET imaging using Pittsburgh Compound-B (PiB) to quantify amyloid burden. Participants with VCI who exhibited PiB uptake 2 standard deviations above the mean of controls were considered to have significant PiB binding and were classified as PiB-positive and to have mixed VCI.

To determine the effect of amyloid pathology on cognitive function we collected the following cognitive measures: 1) ADAS-Cog; 2) EXIT-25; and 3) Three executive processes including a) Digits Forward and Backward Test, b) Stroop Test, c) Trail Making Test (Parts A & B). A Pearson correlation coefficient was used to determine the association between PiB uptake and cognitive function.
Results

Eight out of 18 (44%) participants were PiB-positive and 10 (56%) participants were PiB-negative. PiB-positive and PiB-negative groups did not differ in cognitive functioning (p>0.05), however, increased PiB uptake was associated with greater cognitive dysfunction as measured by the MOCA (r=-0.63, p<0.05) and ADAS-Cog (r=0.61, p<0.05) after controlling for age, gender, and education.

Conclusion

Almost half of the individuals diagnosed with VCI had co-existing amyloid pathology. Critically, increased amyloid was associated with greater memory and executive dysfunction. As such, amyloid deposition plays a key role in cognitive impairments in people with mixed VCI.
Preface

This dissertation is an original intellectual product of the author, D. Dao. The Aging, Mobility, and Cognitive Neuroscience Laboratory assisted with data collection and the UBC Positron Emission Tomography (PET) Imaging Laboratory assisted the data analysis. Ethical approval was obtained from the Vancouver Coastal Health Research Institute (V07-01160) and the University of British Columbia’s Clinical Research Ethics Board (H07-01160).
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1. Introduction

1.1 Cost of dementia

The number of Canadians with cognitive impairment, including dementia, is rising rapidly and poses a significant economic and health care burden. Specifically, the number of Canadians living with cognitive impairment and dementia now stands at 747,000 and will double to 1.4 million by 2040 [1]. Today, the combined direct (medical) and indirect (lost earnings) costs of dementia total $33 billion per year and by 2040, this figure will rise dramatically to $293 billion per year [1]. Canada’s health-care system is ill equipped to deal with these staggering figures.

Alzheimer’s disease (AD) and vascular cognitive impairment (VCI) have been reported as the two most common forms of cognitive impairment [2]. However, these data are typically derived from clinical diagnosis and epidemiological studies that may not have gathered all of the necessary information for accurate diagnosis. Evidence from neuropathological studies suggest that mixed pathologies may be much more common than “pure” forms of dementia – particularly in the case of mixed AD and vascular pathologies [3-8]. In a study of over 1,000 post mortem examinations, of 86% of all those with AD related pathology, only 43% had pure AD and 26% had mixed AD and cerebrovascular pathology [9]. Another autopsy study of 1,110 demented elderly subjects found AD with cerebrovascular lesions to be the second most common pathology (24.7%) after AD (42.9%) [10]. These studies show that a large proportion of individuals with dementia exhibit mixed AD and vascular pathology.

The high prevalence of co-existing cerebrovascular and AD pathology in neuropathological studies suggest that mixed pathologies may be clinically underdiagnosed as a cause of cognitive impairment and dementia [11,12]. Although efforts have been made to recognize mixed AD and vascular pathology [13] in the context of an AD diagnosis, no such efforts have been extended to people with VCI. As such, the
current criteria for the clinical diagnosis of VCI may not distinguish those with cognitive impairment exclusively due to subcortical ischemic small vessel disease from those with cognitive impairment due to mixed vascular and AD pathology. Furthermore, it is unclear how co-existing amyloid pathology, a pathological hallmark of AD, may affect cognitive function in people with VCI – this thesis will refer to people with co-existing VCI and amyloid pathology as mixed VCI. Thus, the purpose of this study was to determine the frequency of mixed VCI in patients clinically diagnosed with VCI. In addition, we investigated how co-existing amyloid pathology may affect cognitive function in people with VCI.

From a public health standpoint, it is critical that we create a proper diagnostic evaluation of mixed VCI as vascular risk factors can be controlled and modified [14]. Those with mixed VCI have worse outcomes and progress more rapidly [15], yet the prevention and treatment of the vascular component of the disease has the potential to markedly reduce the burden of dementia. Critically, strategies to preserve cerebrovascular health may reduce the detrimental effects of dementia avoiding early institutional care and reducing health care costs.

1.2 What is vascular cognitive impairment?

Vascular pathologies leading to dementia have been reported in 8-10% of cognitively impaired older adults and its prevalence in autopsy studies varies from 0.03-58% and averages between 8-15% in western clinical series [16]. Higher rates have been reported in Europe and Asia [17]. An autopsy series of elderly subjects with and without dementia reported prevalence rates between 5-78% and between 4.5-46.8% in the oldest-old [18]. Some of the risk factors associated with cerebrovascular diseases (CVD) include both lifestyle and physiological risk factors. Lifestyle risk factors include low education, low level of physical activity or physical function, increased alcohol intake, smoking, and obesity. Physiological risk factors include history of hypertension, diabetes mellitus, and total cholesterol level [19].
Cognitive impairments associated with vascular pathologies are referred to as “VCI”. Vascular cognitive impairment encompasses the full spectrum of cognitive disorders associated with cerebrovascular disease, from frank dementia to mild cognitive deficits [19]. Vascular cognitive impairment can be associated with both large vessel disease and small vessel disease, including subcortical ischemic vascular disease [20]. This thesis will focus on those with subcortical VCI as this group is suggested to be a more homogenous group of patients that are expected to show greater predictability in their clinical picture, natural history, outcome, and treatment response [21]. Common forms of small vessel damage include small vessel arteriosclerosis, lipohyalinosis, and arteriolosclerosis. Small vessel disease first affects the arteries of the basal ganglia, then expands to peripheral white matter, leptomeningeal arteries, and into the thalamic and cerebellar white matter vessels [22]. Specifically, it is caused by ischemic, ischemic-hypoxic, or hemorrhagic damage to these regions [11]. It is typically associated with more chronic, diffuse, and less severe ischemia causing white matter lesions (WMLs) and lacunar infarcts [23].

White matter lesions in VCI include extensive confluent periventricular and deep lesions mainly affecting the genu or anterior limb of the internal capsule, anterior corona radiata, and anterior centrum semiovale [21]. The few millimeters of white matter adjacent to the wall of the lateral ventricles most frequently become damaged, as they are located at the distal end zone territory of blood supply from the choroidal arteries. This area has been shown to be a low-perfusion region and is very susceptible to ischemic injury from further reductions in blood flow, as evident from the large volume of WMLs in these regions. [24]. It is hypothesized that WMLs in the subcortical and periventricular regions cause cognitive dysfunction by damaging the short looped U-fibers and long association fibers that connect the subcortical nuclei to the cortex, disrupting the connectivity between brain regions [25]. Declines in white matter integrity are associated with general cognitive impairment [26] including declines in information processing speed, executive functions [27], and episodic memory retrieval [28].
Lacunes are small, punctate, deep infarcts that occur in the subcortical regions of the brain caused by occlusion in the deep penetrating blood vessels from “hyalinosis” [29]. In VCI, lacunar infarcts most frequently occur in the caudate, globus pallidus, thalamus, internal capsule, corona radiata, and frontal white matter [21]. Lacunes may damage frontothalamic circuits and have been associated with hypometabolism of the dorsolateral frontal cortex. Multiple lacunar infarcts emphasize frontal lobe dysfunction and are strongly associated with executive dysfunctions [30, 31]. Additionally, lacunes are also associated with memory failure that may arise from frontal lobe dysfunction caused by disruption to frontosubcortical loops [32].

Together, lacunes and WMLs contribute to the emphasis of executive dysfunction in VCI. Executive functions are defined as those higher-order cognitive capabilities that are called upon in order to formulate new plans of action and to select, schedule, and monitor appropriate sequences of action [33]. Thus, it includes many stages necessary for goal-directed behaviour. Executive functions are subserved by frontal brain regions and executive impairment has been attributed to disruptions in the frontal subcortical circuits, which consist of neuronal connections from the frontal cortex to the basal ganglia and thalamus, with feedback from the thalamus back to the frontal cortex [34]. The loss of executive functions include disruptions in frontal-subcortical loops by lacunes in the striatum, globus pallidus, or thalamus or by white-matter lesions that disconnect the prefrontal or anterior cingulate cortices from their basal ganglia or thalamocortical connections [35]; however, dysfunction can occur with damage to any part of the circuit. Damage to these circuits may cause impaired executive control of volition, working memory, organization, language, mood, regulation of attention, constructional skills, motivation, and socially responsive behaviors. This can cause impairment of goal formulation, initiation, planning, organizing, sequencing, executing, set-shifting, set maintenance, abstracting, and cause slowed information processing [36]. Loss of executive functions result in an inability to participate in everyday activities such
as cooking, dressing, shopping, and housework and is a major component of cognitive disability in the clinical picture of VCI [35,37].

1.3 What is Alzheimer’s disease?

Alzheimer’s disease is the most common cause of dementia accounting for 60-80% of dementia cases. Alzheimer’s disease increases dramatically with age, from approximately 53 new cases per 1,000 people age 65-74, to 170 new cases per 1,000 people age 75-84, to 231 new cases per 1,000 people over age 85 [38]. Risk factors for AD include advancing age, family history, apolipoprotein E gene, and traumatic brain injury. Research suggests that vascular risk factors such as diabetes, midlife hypertension, smoking, and raised cholesterol are also associated with the onset of AD [39]. These aggregated cardiovascular risk indices increase the risk for dementia incrementally whether measured in midlife [40] or a few years before dementia onset [39].

Alzheimer’s disease is a neurodegenerative disorder that specifically affects brain regions involved in learning and memory. Pathologically, this disease is characterized by the presence of neurofibrillary tangles (NFT) and amyloid plaques [41]. Neurofibrillary tangles are intracellular aggregates of the microtubule associated protein tau; these structures aid in the transport of molecules within a cell. In AD, tau becomes abnormally hyperphosphorylated causing the microtubules to disintegrate and the communication between neurons to break down [42]. Amyloid plaques are extracellular deposits of aggregated Aβ-peptides (Aβ) [41]. Aβ are created in the proteolytic processing of the amyloid precursor protein (APP). The normal functions of APP are not well understood, however some evidence suggests that it plays an important role in regulating neuronal survival, neurite outgrowth, synaptic plasticity, and cell adhesions. In AD, APP processing involves additional enzymes (β- and Υ-secretase) that cause an increased production and accumulation of Aβ in the brain [43].
It has been suggested that Aβ is the main culprit of AD [44]. The “amyloid cascade hypothesis” [44,45] states that the overproduction of Aβ, or the failure to clear this peptide, leads to AD primarily through amyloid plaque deposition. This hypothesis proposes that the progressive accumulation of Aβ initiates a complex multicellular cascade that includes the production of NFT, microgliosis, astrogliosis, neuritic dystrophy, and neuronal dysfunction and loss. This eventually leads to synaptic insufficiency causing cell death and, ultimately, clinical symptoms such as memory loss and cognitive impairment [46]. Histochemical post-mortem brain examinations show an initial diffuse distribution of amyloid within the neocortex that progresses towards the temporal allocortex, including the hippocampus as well as subcortical brain structures during the course of the disease [47]. In the clinical stages, there is a higher concentration of amyloid plaque deposit in neocortical regions including the frontal, parietal, and lateral temporal cortices as well as the posterior cingulate and the striatum [48].

Brain regions ravaged by Aβ typically exhibit reduced numbers of synapses and neurites. Synapses may be particularly susceptible to the adverse effects of Aβ, as it has been shown to impair synaptic ion and glucose transporters and electrophysiological studies show that Aβ may impair synaptic plasticity [49]. Aβ may also damage neurons by disrupting both pre- and postsynaptic terminals by inducing oxidative stress, impairing calcium homeostasis, and perturbing the functions of mitochondria and the endoplasmic reticulum. These processes are the cause of major alterations involved in the functional and structural abnormalities of synapses and axons in AD [41].

Clinically, AD first manifests as mild cognitive impairment (MCI) in which deficits in episodic memory appear to be most effective at identifying individuals at risk for transitioning to AD [50]. Episodic memory involves conscious retrieval of information acquired at a particular place and time [51] and is one of the salient features of AD [52]. This is not surprising as AD pathology is initially selective for limbic regions that subserve episodic memory and the earliest neurofibrillary changes occur in medial temporal
lobe structures (e.g., hippocampus and entorhinal cortex), interrupting the neural network critical for episodic memory function [53]. Episodic memory impairment in AD manifests as forgetfulness and the inability to learn and remember new information (i.e., anterograde amnesia). In addition, studies have shown that patients with AD are impaired on free recall, recognition, and paired-associate learning. These deficits have been attributed to ineffective consolidation or storage of new information rather than to a deficit of retrieval [54].

Alzheimer’s disease patients also show deficits in semantic memory or the general knowledge of facts, concepts, and the meanings of words. Semantic memory deficits reflect the spread of pathology to the temporal neocortex [55]. Specifically, patients with AD are often impaired on tests of object naming, verbal fluency, and semantic categorization [54]. Furthermore, this loss is specific to the deterioration in the structure of semantic memory rather than a general inability to retrieve or access semantic knowledge [56]. For example, patients with AD are more impaired on category fluency (e.g., generating lists of animals) compared with phonemic fluency (e.g., generating words beginning with a specific letter) [57]. Knowledge for particular items or concepts and the associations between them may also be disrupted as the neuropathology of AD spreads from the temporal to the frontal and parietal association cortices [54].

People with AD also show deficits in executive functions [54]. Compared with controls, mildly demented AD patients are significantly impaired on tests that require set shifting, self-monitoring, or sequencing, but not on tests that require cue-directed attention or verbal problem solving [58]. With disease progression, working memory, attention, and visual-spatial abilities also become significantly impaired and there is a complete loss of executive functions resulting in an inability to participate in activities of daily living [54].
1.4 Interaction between vascular cognitive impairment and Alzheimer’s disease

1.4.1 Pathophysiology

Previously, AD and VCI were considered to be distinct and unrelated. However, the traditional strict dichotomization between these two types of dementia has recently been challenged. Diagnostic criteria typically classify dementia as either vascular or AD-driven, yet the reality of clinical practice show that vascular comorbidity may be present in 30%-60% of AD patients [59] and AD pathology may be present in 40%-80% of vascular dementia patients [60]. Furthermore, studies have found greater WML volume in people with AD compared with normal aging adults [61] and over 60% of older patients with AD present with white-matter infarction [62]. The high prevalence of co-existing vascular and AD pathology may be attributed to the fact that VCI and AD share common vascular risk factors such as hypertension, arterial disease or atherosclerosis, stroke, hyperlipidemia, ischaemic heart disease, smoking, and diabetes mellitus [19]. These vascular pathologies may cause localized or global hypoperfusion, which may lead to both Aβ accumulation and WMLs [63].

Abnormalities in the microvascular system of the brain may contribute to the pathogenic changes in AD including decreased cerebral perfusion, a reduction of glucose transport and utilization, the loss of vascular innervation (i.e. cholinergic transmitters), impairment of neurogenic cerebrovascular regulation, ultrastructural changes in capillaries and basement membranes, and breakdown of the blood–brain barrier [3]. These changes may also disrupt the neurovascular unit of the brain - the neurovascular unit refers to the interactions among glial, neuronal, and vascular elements [63]. The hemodynamic communication between neurons and the cerebrovasculature is necessary for proper brain function and impairment of this system is increasingly linked to diseases of the central nervous system [3,64,65].

There is evidence suggesting that a disrupted functional relationship between cells in the neurovascular
unit is an early event in AD pathogenesis [64]. Breakdown of the neurovascular unit can disrupt the blood brain barrier and compromise the structural and functional integrity of the brain. An important function of the blood-brain barrier is to regulate the movement of Aβ between the brain, plasma, and cerebrospinal fluid. Typically, the concentration of Aβ in these three compartments is in equilibrium and the influx of soluble Aβ across the blood brain barrier is modulated by its interaction with the receptor for advanced glycation end products (RAGE) and the efflux of Aβ is controlled by the low-density lipoprotein receptor on brain endothelial cells. Accumulating evidence from patients and animal models of AD suggests that AD brains may suffer from an increase in influx receptors (RAGE) and/or a decrease in efflux receptors (lipoprotein receptor-related protein) causing greater Aβ accumulation within the brain [66]. This suggests that cerebral ischemia may be a powerful modulator in amyloidosis.

In addition, the accumulation of Aβ may have negative consequences on the functioning of the cerebral vasculature. For example, amyloidosis is thought to be a powerful modulator in cerebral metabolic function. Typically, there is a homeostatic mechanism that matches brain activity with substrate delivery via increases in cerebral blood flow (CBF). Specifically, there is a close relationship between CBF and cerebral glucose utilization (CGU), a variable reflecting neural activity. In the resting brain, regions with high CGU also have high CBF and vice versa. This property of cerebral circulation assures that different regions of the brain receive sufficient oxygen and glucose to meet their functional needs [64]. Studies with APP transgenic mice exhibiting an overexpression of APP and Aβ demonstrate disruptions in the relationship between neural activation, CBF, and CGU. For example, APP transgenic mice exhibit decreased CBF in response to functional activation produced by somatosensory stimuli and this attenuation correlates strongly with the amount of brain Aβ [67]. Furthermore, resting CBF was found to be reduced in brain regions affected by AD such as the parietal and temporal cortex, and in the hippocampus and amygdala. This reduced CBF is coupled with reduced CGU in mice with higher Aβ levels [68]. The relationship between neuronal activation, CBF, and CGU was altered before the
formation of amyloid plaques. These data indicate that Aβ can exert powerful effects on cerebrovascular regulation early in the disease process and before the formation of mature amyloid plaques and neurodegeneration.

In addition to its neural toxic effects, Aβ also causes vascular dysregulation by disturbing cerebrovascular regulation [69]. It has been suggested that APP overexpression and Aβ produce cerebrovascular alterations by inducing oxidative stress and inflammatory mediators [70]. Aβ may also threaten cerebrovascular function by compromising cerebral perfusion, reducing vascular reserves, and increasing the propensity for ischemic damage [63]. In return, hypoxia and/or ischemia may promote the cleavage of Aβ from APP by up-regulating β- and Y-secretase activity [71]. Furthermore, studies have found that focal cerebral ischemia produces larger infarcts in mice overexpressing APP [72,73]. Human studies have found brain Aβ to be elevated in patients with vascular dementia [74] and patients with AD have a heavier burden of cerebrovascular lesions compared with normal controls [65]. These studies suggest that there is a positive feedback loop effect between Aβ and cerebrovascular dysfunction.

In summary, multiple pathogenic cascades in the neurovascular unit may contribute to both VCI and AD pathology. Disintegration of vascular integrity can cause WMLs and lacunes and promote amyloidosis through compromised neuronal metabolism, mitochondrial deficiency, protein degradation failure, oxidative stress, and inflammation [65]. Moreover, both Aβ and vascular risk factors target the structure and function of cerebrovascular cells, glia, and neurons (neurovascular unit), resulting in neurovascular dysfunction [63]. Taken together, Aβ has been shown to have damaging effects on cerebrovascular function, while cerebral ischemia is thought to be a powerful modulator in amyloidosis [75] suggesting a close interaction between AD and VCI pathology.
1.4.2 Cognitive function

With age, VCI and AD commonly occur together and each may contribute to the progression of dementia [10]. The coexistence of ischemic and neurodegenerative pathology was found to have a profound impact on the expression of dementia, suggesting reciprocal interactions between ischemia and neurodegeneration [76]. Current evidence suggests that the presence of ischemic lesions is associated with greater degree of cognitive deficits among patients with AD [77-79].

Concomitant cerebral infarcts such as basal ganglia lacunes and WMLs have been associated with greater cognitive dysfunction in people with AD [78,80]. Furthermore, vascular risk factors such as total cholesterol, low-density lipoprotein, and diabetes are associated with accelerated cognitive decline [81]. In addition, studies report that cerebrovascular lesions may lower the threshold of AD pathology required for the development of dementia [77]. Specifically, the effect of vascular lesions is more pronounced in patients in the early stages of AD [77] – it is hypothesized that vascular lesions may magnify the effect of mild AD and result in more severe cognitive impairment [80]. Thus, coexisting CVD or incident ischemic lesions may shorten the preclinical stage of AD and accelerate disease progression.

Typically, individuals with AD show greater impairment on measures of episodic memory and patients with VCI show greater executive dysfunction [55]. Memory deficits in VCI are typically milder compared to AD. People with VCI have a relatively intact recognition memory, less severe forgetting, and benefit more from cues compared to those with AD [82]. In addition, people with vascular forms of dementia show greater attentional deficits, lower frequency of intrusion errors, and lower level of language dysfunction compared to people with AD [55]. Differences on neuropsychological performance can be attributed to differences in the underlying pathophysiology of each disease. Currently, it is unclear how co-existing amyloid and vascular pathologies may impact cognitive function. Specifically, it is unknown
how co-existing vascular and amyloid pathology may uniquely contribute to cognitive dysfunction and how it may differ from symptoms of pure AD and VCI.

There is consensus that cognitive function and global executive function measures, such as the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-Cog) [83] and the Executive Interview Test (EXIT-25) [84], must be included as part of the optimal assessment battery in AD and VCI trials respectively [85]. The ADAS-Cog is sensitive to a wide range of disease severity and is specific to the central dysfunctions experienced by patients with AD including memory, praxis, and language. It is regarded as the standard instrument for use in clinical trials as a primary index of cognitive change in AD [86]. The EXIT-25 provides a standardized clinical assessment of executive control functions. Items assessed include verbal fluency, design fluency, frontal release signs, motor/impulse control, imitation behavior, and other clinical signs associated with frontal system dysfunction [84]. These tests were developed to tap into cognitive domains specific to either AD or VCI and care must be taken in extrapolating AD-specific evaluations to VCI and vice versa – important differences in specific domains affected and characteristics of disease course must be taken into account. As such, additional tests of specific executive functions, such as working memory (Digits Forward and Backward Test), attention and response inhibition (Stroop Test), and set shifting (Trail Making Test) should also be included [22]. Vascular and degenerative pathologies may interact to promote cognitive dysfunction and the nature of these inter-relations and the relative contribution of neurodegenerative pathology, such as amyloid plaques, to cognitive impairments in VCI require further investigation. Understanding the domains of cognitive dysfunction in mixed amyloid and VCI pathology may be a useful adjunct to the correct diagnosis of mixed VCI.
1.5 Neuroimaging and dementia

One of the greatest clinical challenges of dementia is establishing an approach that would ensure the early identification and accurate diagnosis of dementia subtypes, thus, making it possible to treat or delay the progression of the disease. Currently, there is great effort invested in the use of neuroimaging to identify potential biomarkers that would distinguish between dementia subtypes. Positron emission tomography (PET) is a potential tool to differentiate mixed pathologies from pure AD and VCI.

1.5.1 PET instrumentation

PET is a nuclear imaging technique that uses radioactive tracers to investigate tissue function in vivo. PET images are acquired by detecting the decay of positron-emitting radioisotopes which can either be inhaled or injected into the subject. Ideally, the tracer is rapidly taken up into the circulation system and distributed to the target location or binding site. As the radioactive tracer decays, it releases a positron that will collide with an atomic electron in its immediate vicinity and cause an annihilation reaction. This reaction converts the mass of the electron and the positron into electromagnetic energy. Specifically, two high-energy gamma rays (photons) are emitted simultaneously in opposite directions (180 degrees apart) carrying an energy of 511 keV. Positron emission tomography imaging utilizes the inherent characteristics of these annihilation photons. First, these high-energy photons have a high probability of escaping the body. Second, these photons are emitted with a precise geometric relationship, thus they can be detected and localized (the line joining the two photons, termed the line of response (LOR), passes directly through the point of annihilation). A PET scanner is designed to detect and localize these annihilation photons.

To detect a pair of annihilation photons a PET scanner is comprised of detectors efficient at identifying/stopping 511 keV photons. These detectors are called scintillators, which are made from a
dense crystalline scintillating material. The scintillator heads are organized in a geometrical cylinder surrounding the gantry and are attached to photomultiplier tubes. The scintillator serves as an interacting medium for the gamma rays. As the gamma rays interact with the scintillator material it emits visible light, which will then pass through a photomultiplier tube. The photomultiplier tube then converts the scintillator photons into an electrical signal.

To create an image, the photons must be detected in coincidence. That is, the two photons must be detected within a short time window to ensure that they originated from a single annihilation event. In addition, the energy detected by the photons must lie within a specific energy window so that photons that have scattered in the body are rejected. The annihilation events are recorded in such a way that information about the energy and timing of the photon can be derived. Events that meet both the energy and timing criteria are then sent to sorting hardware that writes the raw data into a histogrammed 2-D matrix known as a sinogram. Data from the sinogram give information about the LOR, which traces the path of the annihilation photons. This information can be used to localize the annihilation event to create a 3-D image [87].

1.5.2 Issues with PET data collection

Producing accurate PET images relies on the accuracy of coincidence detection; however, accurate detection of coincidence events may not always be possible. There are several physical phenomena that would result in the incorrect positioning of events and cause image degradation. They are discussed in the sections below.

*Positron range and non-collinearity*

There are two effects in PET imaging systems that lead to errors in determining the location of the
positron emission. One effect is positron range – the distance from the site of positron emission to the site of annihilation. A positron can undergo multiple direction changes as it interacts with other electrons prior to annihilation. A PET scanner is set up to detect the annihilation photons, which define the line along which the annihilation takes place, not the line along which the decaying atom is located. The second issue is that the positron and electron are not completely at rest when they annihilate which means that the annihilated photons will not be exactly 180 degrees apart. This is known as non-collinearity. These two effects can cause a blurring in the reconstructed images degrading spatial resolution [87].

Detection of scattered events

Photons created by annihilation reactions may interact with other matter. Compton scattering is the major mechanism by which the photons may scatter in matter. In Compton scattering, the 511 keV photons may scatter off a free or loosely bound electron transferring some of its energy to the electron and changes direction in the process. Compton scattering in the body attenuates the signal by redirecting annihilation photons that would have otherwise struck a particular detector pair.

To ensure that the photons have not scattered the following criteria may be enforced: 1) the two photons are detected within a predefined electronic time window (to ensure that the detected photon pair resulted from the same annihilation event); and 2) The energy deposited in the crystal by both photons is within the selected energy window (to ensure that the photons have not interacted with the surrounding atoms in a significant way). The most accurate scatter correction method are simulation-based methods in which scatter coincidences can be mathematically corrected in the images [87].

Detection of random events

A random (accidental) coincidence occurs when two separate annihilation events occur and one photon
from each separate annihilation event is mistakenly considered a true coincidence. This can occur if one photon is propagated outside the field of view or if the detectors fail to detect it (e.g. due to dead-time or pile-up). This will result in incorrect positioning of the LOR. Random coincidences are a direct consequence of utilizing a large coincidence timing window – allowing two unrelated photons to be temporally close enough to be recorded within the coincidence timing window. To correct for this, the coincidence window can be shortened; however, this can also result in loss of sensitivity to true coincidences. The most common method of correcting for random coincidences is the delayed channel method. Here timing signals from one detector are delayed by a time significantly greater than the timing window used to capture a true coincidence. There will therefore be no true coincidences in the delayed coincidence channel and the number of coincidences recorded is a good estimate of the number of random coincidences in the collected data [87].

**Attenuation**

Attenuation is the loss of detection of true coincidence events because of their absorption in the body or due to photons scattering out of the detector’s field of view. Loss of counts due to attenuation increases image noise, image artifacts, and image distortion. Without attenuation correction, significant artifacts include: 1) prominent activity at body surface edges due to relative lack of attenuation at the surfaces compared to deeper structures; 2) distorted appearance of areas of intense activity due to variable degrees of attenuation in different locations; and 3) diffuse or relatively increased activity in tissues of relatively low attenuation (e.g. lungs).

Fortunately, attenuation effects can be easily corrected. The attenuation of gamma rays depends on the total thickness of the attenuating material; thus, attenuation can be corrected for by measuring the thickness of the attenuating material. This can be achieved by performing a transmission scan prior to the
emission scan. A transmission scan is performed using an external positron-emitting rod located in the gantry of the scanner with (transmission scan) and without (blank scan) the patient in the scanner. Essentially, the count rate during the transmission scan is compared to the blank scan to yield a transmission coefficient that can be used to mathematically correct the image. It essentially “adds counts back” into areas that are more attenuated due to it being deeper or being surrounded by relatively dense structures and “subtracts counts” from areas that are less attenuated. Attenuation correction is the largest correction made to PET image data [87].

**Normalization**

Non-uniformities in individual detector efficiencies (e.g., position of the elements in the block, physical variations in the crystal and light guides, and variation in the gains of the photo-multiplier tubes), geometrical variations (e.g., a photon entering a crystal at an angle will usually have more material in its path compared to ones entering at right angles, and thus having an increased probability of interaction), and detector electronics (e.g., energy thresholds, time window alignment) all contribute to variations in coincidence detection efficiency between different LORs (i.e., pairs of detector elements) in the system. Normalization corrects each individual LOR for these non-uniformities. To correct for this, a normalization scan is performed. A source with a known number of emissions is scanned and compared to the number of detected emissions and a correction factor is calculated for a normalization correction. This method will directly measure the relative variation in coincidence detection efficiencies between all the LORs in the system [87].

**Other corrections applied to PET imaging**

In addition to the above-mentioned corrections, the following complications must also be accounted for. This includes decay and dead-time corrections. Decay correction accounts for the decaying properties of
the radioisotope as the tracer is decaying at an exponential rate over time. Thus, as the scan progresses there will be fewer annihilation photons and fewer reconstructed counts in the image. Decay correction involves scaling the reconstructed image counts and is dependent on the half-life of the radioisotope used. Dead-time is the time it takes to process a coincidence event, during which no other coincidences can be processed. Several components in the detection chain will experience some level of dead-time as each subsystem will require a minimum amount of time to pass for each event to be registered as separate. The main source of dead-time in most PET systems comes from the processing of events in the detector front-end electronics. This dead-time is mainly dictated by the extent of signal integration necessary for accurate energy discrimination and event positioning in the detector module. Other contributions to dead-time can come from the coincidence event processing, real-time sorting of data into sinograms, and data transfer. Correction for dead-time typically involves a model of the dead-time behavior of the system at different count rate levels. Because only small doses of tracer are administered, dead-time counts are minimal and only a small correction is necessary [87].

1.5.3 Amyloid imaging

PET tracers must meet several requirements to be successfully used. First, a sufficient amount of the tracer should be able to cross the blood brain barrier and enter the brain. This amount should also enter the brain over a reasonable time period. Secondly, the imaging agent should have sufficient affinity for the target so that a large fraction of the imaging agent remains bound to the target over the period of time required for the majority of the free and non-specifically bound imaging agent to clear from the brain. Furthermore, the clearance of the majority of the free and non-specifically bound imaging agent should occur over a period of time that is practical (this may be as little as 15-60 min for an 11C labeled PET tracers) [88,89].
Currently, the most popular radioactive tracer to image amyloid is Pittsburgh compound B (PiB). Development of PiB first required the modification of the amyloid binding histological dye, thioflavin-T. This led to the discovery that neutral benzothiazoles, specifically 2-(4’-Methylaminophenyl)benzothiazole (BTA-1), crossed the blood–brain barrier and bound to amyloid plaques with high affinity [90]. BTA-1 could bind to amyloid plaques with low nanomolar affinity, enter brain in amounts sufficient for PET imaging, and clear rapidly from normal brain tissue. At the low nanomolar concentrations typically used in PET studies, the binding of BTA-1 in postmortem human brain was shown to bind specifically to amyloid plaques, and importantly did not bind to NFT [91]. Further studies found that a hydroxylated BTA-1 derivative had brain clearance properties typical of many useful PET radiotracers [92]. These innovations eventually led to the development of N-methyl [11C] 2-(4’-methylaminophenyl)-6-hydroxy-benzothiazole, more commonly known as PiB. PiB attaches itself to the amyloid ‘receptor’. This ‘receptor’ is a polymer composed of Aβ peptide subunits that forms the amyloid fibril. The polypeptide backbone of each Aβ peptide in the fibril is folded into a β-pleated sheet or a cross β-structure. The unique stereochemistry of this β-pleated sheet allows the fibrils to bind specifically to PiB. As such, PiB attaches specifically to fibrillar amyloid (amyloid plaques) and not diffuse amyloid (oligomeric species) [93]. Currently, PiB is the most validated and popular radioactive tracer to image amyloid plaques [94-98].

1.5.4 Use of PiB-PET imaging in dementia

Although the exact cause of AD has not been determined, the amyloid cascade hypothesis remains the best-defined and most studied conceptual framework for AD. Supporting evidence for the amyloid cascade hypothesis has come from divergent fields of research including genetics, histopathology, cell biology, and animal models [44,99,100]. The strongest evidence linking AD to the production of Aβ comes from genetic studies. Alzheimer’s disease has been linked to genetic mutations in the APP, PS1, and PS2 genes and these defected genes have been shown to cause an increase in Aβ production in
patients, cultured cells, and transgenic mice [101]. In addition, inheritance of apolipoprotein E-ε4 (APOE-ε4) gene is the most significant risk factor for developing AD and the presence of APOE-ε4 has been correlated with increased Aβ deposition [102].

Several PET studies using PiB as the binding agent for amyloid plaques have shown that levels of PiB retention can be used to differentiate between patients with AD and age-matched, healthy individuals with normal cognitive function. Studies have shown significantly higher cortical PiB retention in AD subjects compared with controls [48,95,103,104]. Additional studies have shown that PiB retention largely remains stable in patients with AD [105] and that plaque levels plateau before the onset of clinical symptoms [106]. This evidence suggests that PiB retention might be a potential biomarker for the early diagnosis of AD [107].

Based on global brain PiB-PET uptake, subjects are often dichotomized into groups with high and low PiB uptake values – PiB-positive and PiB-negative groups [108-110]. Data from 15 research groups have shown that 96% of 341 clinically-diagnosed AD patients were PiB-positive [111]. Furthermore, Okello and colleagues [112] found that 17 of 31 (55%) subjects with MCI had increased PiB retention at baseline and 14 of those 17 (82%) clinically converted to AD at follow-up. Only one of the 14 MCI PiB-negative subjects converted to AD. PiB-PET measurements have also been confirmed by autopsy studies that have shown a strong direct correlation of in-vivo PiB retention with region-matched quantitative analyses of amyloid plaques in the same subjects post mortem [94]. These findings highlight the diagnostic sensitivity of PiB and support the validity of PiB-PET imaging as a method for *in-vivo* evaluation of amyloid plaque burden. As such, PiB-PET imaging shows great potential in the differential diagnosis of dementia subtypes. Specifically, PiB-PET imaging of amyloid plaques will allow the detection of AD pathology in people with VCI for the identification of mixed VCI.
1.6 Study objectives and hypotheses

The purpose of this study is to determine the frequency of mixed VCI using PiB-PET imaging in patients clinically diagnosed with VCI. In addition, we will investigate how co-existing amyloid plaques may affect cognitive function in people with VCI.

Based on the literature, the following hypotheses were proposed:

1. A subset of individuals with VCI will display co-existing amyloid pathology and have mixed VCI.

2. Greater amyloid plaque deposition will be associated with greater cognitive dysfunction as measured by following cognitive measures: 1) ADAS-Cog; 2) EXIT-25; and 3) Three executive processes including: a) Digits Forward and Backward Test b) Stroop Test c) Trail Making Test (Parts A & B).
2. Methods

2.1 Ethics statement

Ethical approval was obtained from the Vancouver Coastal Health Research Institute (V07-01160) and the University of British Columbia’s Clinical Research Ethics Board (H07-01160). All participants provided written informed consent.

2.2 Study protocol

This is a cross sectional analysis of data acquired from a randomized controlled trial investigating the effects of aerobic training in people with VCI (ClinicalTrials.gov number NCT01027858) \[113\]. Those who met the eligibility criteria and consented to PET imaging were recruited to take part in this sub-study. Before the start of the trial, participants completed baseline tests of cognitive function, global executive function, and key executive processes. A blinded research associate performed all cognitive testing. Within two months of completing the exercise intervention trial, participants underwent a PiB-PET scan and a T2 MRI scan for co-registration with PET images. Healthy controls were recruited as a comparison group for PiB-PET analysis.

2.3 Power

As this was a pilot study, a power analysis was not performed.

2.4 Participants

VCI Participants: A study neurologist screened interested participants to determine if they met the diagnostic criteria for VCI as outlined by Erkinjuntti and colleagues \[114\], which requires the presence of both small vessel ischemic disease and cognitive syndrome. A research coordinator then determined if
participants met the inclusion and exclusion criteria for the research study. A total of 18 participants (8 females and 10 males) were determined to meet the study criteria and consented to take part in this substudy. Details are described below.

Small Vessel Ischemic Disease: Evidence of relevant cerebrovascular disease by brain imaging defined as the presence of both: 1) Periventricular and deep WML: patchy areas of low attenuation or diffuse symmetrical areas of low attenuation with ill defined margins extending to the centrum semiovale, plus at least one lacunar infarct. 2) Absence of cortical and or cortio-subcortical non-lacunar territorial infarcts and watershed infarcts, hemorrhages indicating large vessel disease, signs of normal pressure hydrocephalus, or other specific causes of WML (i.e. multiple sclerosis, leukodystrophies, sarcoidosis, brain irradiation).

Cognitive syndrome: defined as: 1) Dysexecutive syndrome: some impairment in goal formulation, initiation, planning, organizing, sequencing, executing, set-shifting and maintenance, or abstracting. 2) Memory deficit: some impairment in recall, relatively intact recognition, less severe forgetting, and benefit from cues.

Inclusion criteria: Montreal Cognitive Assessment score less than 26 at screening [115]; Mini-Mental State Examination score of greater or equal to 20 at screening [116]; lives in Metro Vancouver; must be able to read, write, and speak English; if participants are on cognitive medications (i.e. donepezil, galantamine, rivastigmine, memantine, etc.) they must be on a fixed dose for the duration of the trial; must be in sufficient health to participate in the study’s aerobic-based exercise training program; and provide informed consent.
**Exclusion criteria:** Absence of small vessel ischemic lesions such as WMLs or lacunes on brain CT or MRI; diagnosed with another type of dementia (eg, AD, dementia with lewy bodies, or frontal temporal dementia) or other neurological conditions (eg, multiple sclerosis or Parkinson’s disease); taking medications that may negatively affect cognitive function (eg, anticholinergics); and people who plan to participate in a clinical drug trial concurrent to this study.

**Normal Controls:** The normal control group consisted of volunteers recruited from the community via newspaper ads. Inclusion criteria included MOCA score of 26 or greater, no history of neurologic or psychiatric illnesses, and no abnormalities on neurologic examination.

2.5 Measurements

**Scanning Protocol:** The PET scans were performed using carbon labeled PiB (11C-PiB) produced at UBC TRIUMF. Scans were collected in 3-D mode using the GE Advance tomograph (General Electric, Canada/USA). Prior to injection, a 10-minute transmission scan with a $^{68}$Ge rod was collected for attenuation correction. After the transmission scan, 555 to 560 MBq of 11C-PiB was injected as a bolus into an antecubital vein and flushed with saline. A 90-minute dynamic acquisition immediately followed injection. Thirty-five slices of 4.25-mm thickness that span the entire brain were collected.

**MRI Acquisition:** MR images were acquired on a 3T Intera Achieva MRI scanner (Philips Medical Systems Canada, Markham, Ontario, Canada). High-resolution 3D T1 anatomic images (TR = 8 ms, TE = 3.7 ms, bandwidth = 2.26 kHz, voxel size = $1 \times 1 \times 1$ mm) were collected for co-registration with PET images in the VCI sample.
**Cognitive Function:** Cognitive function was assessed using the cognitive section of the Alzheimer Disease Assessment Scale (ADAS-Cog). This scale assesses memory, language, and praxis. There are 11 tests and scores range from 0 to 70 with higher scores indicating greater cognitive dysfunction. The ADAS-Cog has marked advantages as an outcome measure, based on the substantial data confirming both its reliability and validity and its use in measuring longitudinal change together with sensitivity to treatment effects [117]. The inter-rater reliability of the ADAS-Cog is 0.989 [118] and its test-retest reliability is 0.915 [118].

**Global Executive Function:** Global executive dysfunction was assessed using the EXIT-25 [84,119]. This is a standardized clinical assessment of executive functions and is designed to detect signs of frontal system pathology. This test contains 25 items and scores range from 0 to 50 with higher scores indicating impaired global executive function. This measure has been found to be sensitive in detecting executive deficits in people with mild dementia [119] and to accurately separate non-demented subjects from those with cortical or subcortical dementias [37]. In addition, the EXIT-25 has been significantly associated with frontal brain damage [120]. Its inter-rater reliability is 0.90 [121].

**Key Executive Processes:** Three executive processes were measured by standard neuropsychological tests including: 1) Digits Forward and Backward Test – a measure of updating/working memory [122]. Both test consist of seven pairs of random number sequences that is read out loud to the participant at a rate of one per second. The sequence begins with a three digit pair and incrementally increases by one digit up to a length of nine digits. The participant is asked to remember the sequence of numbers and recite them out loud in either the forwards or backwards order. Testing is complete when the participant fails to recollect any pair of sequences. Digits test score was calculated as the difference between the verbal digits span forward and backward scores – smaller difference scores indicated better working memory. 2) Stroop Test – a measure of selective attention and conflict resolution. The Stroop test involved three different
conditions. First, participants were asked to read out words of colours printed in black ink (eg, yellow). Second, they were asked to read out the colour of coloured x’s. Lastly, they were shown a page with colour words printed in incongruent coloured inks (eg, the word yellow printed in red ink) and were asked to name the colour of the ink in which the words were printed (while ignoring the word itself). There were 80 trials for each condition, and the time it took to complete each condition was recorded. Stroop score was calculated as the time difference between the third and second condition – smaller time differences indicate better selective attention and conflict resolution. [123]. The reliability and validity of these measures have been confirmed [124]. 3) Trail Making Test (Part A and B) – a measure of set shifting [125]. Part A assesses psychomotor speed and requires the participant to draw lines to connect encircled numbers in a numerical order (eg, drawing a line from 1 to 2, 2 to 3, and 3 to 4 and so on). Part B consists of encircled numbers and letters. Participants were asked to draw a line going from a number to a letter in ascending order as quickly but as accurately as possible (eg, 1 to A, A to 2, B to 3, and so on). Trails score was calculated as the difference between Part B and Part A completion times – smaller difference scores indicated better set shifting.

2.6 Data analysis

2.6.1 Imaging analysis

PiB-PET Data Analysis: Parametric images of the binding potential were generated using Logan analysis [126,127] of the full dynamic PET data, with the cerebellum as the reference region. Logan graphical analysis uses a noniterative solution to a multicompartamental model for tracer behaviour. The result is a “slope” that is representative of the tracer distribution volume in the target tissue compared to the reference tissue. Specifically, this method uses the reference region as a measure of non-specific and free tracer concentration. This value is assumed to be proportional to the nondisplaceable tracer concentration in the target tissue and is used to calculate specific binding in regions of interest. As such, it is important that the reference tissue contains minimal specific binding and the cerebellum has been found to contain a
negligible about of amyloid plaques [128]. To express regional binding values, the binding potential which refers to the ratio at equilibrium of specifically bound radioligand to that of nondisplaceable radioligand in tissue (BP_{ND}) [129], was calculated by the equation \(\text{BP} = \text{DVR} - 1\). This method has been validated as reliable for quantification of amyloid plaque deposition [96,130].

*Regions of Interest Analysis in VCI Sample:* Using SPM 8 (Wellcome Department of Cognitive Neurology, Institute of Neurology, University College London) each subject’s MRI image was co-registered to the corresponding mean PiB-PET image. A normalization to the T1 MRI template (in MNI space) was calculated and applied to the co-registered MRI image as well as the PiB-PET data (mean and parametric images).

A custom set of regions of interest (ROIs) were defined on the coronal view of the MNI305 template [131]. These ROIs were transposed to each subject’s warped MRI and PET images (in MNI space). ROIs were adjusted as necessary using both the MRI and mean PiB-PET image for guidance (1-2 pixels maximum). The modified set of ROIs was applied to the parametric PiB-PET image and the average BP_{ND} within each ROI extracted.

Global PiB uptake was determined by averaging BP_{ND} values in the bilateral frontal (combined orbitofrontal and medial prefrontal cortex), parietal (combined angular gyrus, superior parietal, precuneus, and supramarginal gyrus), temporal (combined lateral temporal and middle temporal gyrus), and occipital cortices, and anterior and posterior cingulate gyrus.

*Regions of Interest Analysis in Control Sample:* MRI scans were not collected for control subjects. Thus, a healthy PiB-PET image template was created in MNI space to accommodate normalization of healthy...
PiB-PET images to the common space. Briefly, a series of healthy PiB-PET scans were normalized to one-another and then an average image was created. This PiB-PET image template was then normalized to the T1-MRI template. Individual PiB-PET images (mean and parametric) were then normalized to the MNI space via this healthy PiB-PET image template, allowing the use of the pre-defined ROI template. BP\textsubscript{ND} values were extracted and the global PiB uptake was calculated using the method described above.

2.6.2 Statistical analysis

All statistical analyses were performed using Statistical Package for the Social Sciences 17.0.

Frequency of Mixed VCI: Patients with VCI were classified as either PiB-positive or PiB-negative according to measured global PiB uptake values. Participants were considered PiB-positive if their global PiB uptake value was more than 2 standard deviations above the mean of the normal controls. PiB-positive VCI patients were considered to have mixed VCI [109].

Amyloid uptake and cognitive function: To compare differences in cognitive function between PiB-positive and PiB-negative groups we conducted a student’s t-test. To determine the associations between PiB uptake and cognitive function we conducted a correlational analysis using the Pearson correlation coefficient. BP\textsubscript{ND} values were correlated with cognitive scores on the ADAS-Cog, EXIT-25, Digits Forward and Backward Test, Stroop Test, and Trail Making Test after controlling for age, gender, and education. To further investigate significant correlations, a multiple linear regression model was constructed to determine the independent contribution of PiB uptake on cognitive performance. Age, gender, and education were statistically controlled for by entering these three variables into the regression model first.
3. Results

Participant Characteristics: Within the control sample (a total of 6 participants – 2 females and 4 males), the mean age was 55.3 ± 17.6 and the average MOCA score was 27.3 ± 1.5. The average global BP_{ND} of the control group was -0.12 ± 0.04. Within the VCI same (a total of 18 participants – 8 females and 10 males), the mean age was 74.1 ± 6.3 years with an average MMSE score of 27.8 ± 2.0 and an average MOCA score of 23.8 ± 2.4. The overall BP_{ND} in the VCI sample was 0.07 ± 0.23. The average global BP_{ND} for the PiB positive group was 0.27 ± 2.8 and the average for the PiB negative group was -0.08 ± 0.05. PiB positive and PiB negative groups did not significantly differ in age, MMSE score, functional comorbidity index (a measure of total number of comorbidities), and educational attainment. Detailed demographic characteristics, neuropsychological test results, and PiB retention values are presented in Table 1.

Frequency of Mixed VCI: Of 18 participants, 8 participants (44%) clinically diagnosed with VCI were PiB-positive and 10 participants (56%) were PiB-negative (Figure 1).

Amyloid uptake and cognitive function: There were no significant differences between PiB-positive and PiB-negative groups on tests of cognitive function (p>0.05 – Table 1). However, PiB uptake was significantly correlated with the MOCA (r=-0.63, p=0.01 – Figure 2) and ADAS-Cog (r=0.61, p=0.01 – Figure 3) after controlling for age, gender, and education – increased PiB uptake resulted in increased cognitive dysfunction as measured by MOCA and ADAS-Cog (Table 2). Specifically, the ADAS-Cog showed measures of memory and praxis to be significantly correlated (r=0.55, p=0.04) with PiB retention with measures of language trending towards a significance (r=0.47, p=0.08). In addition, a linear multiple regression showed PiB uptake to be independently associated with MOCA (R-Square Change = 0.39, F
Change = 8.59, Sig F Change = 0.012 – Table 3) and ADAS Cog (R-Square Change = 0.36, F Change = 7.82, Sig F Change = 0.015 – Table 4) after accounting for age, gender, and education.
4. Discussion

This is the first study to assess the frequency of mixed VCI in people clinically diagnosed with VCI within a North American population. Furthermore, we included a detailed neuropsychological evaluation of cognitive domains specifically affected by both AD and VCI. Our results indicated that 44% of people clinically diagnosed with VCI had abnormal levels of amyloid plaques. Importantly, increased PiB retention was associated with increased cognitive dysfunction as measured by the ADAS-Cog and MOCA. Thus, amyloid deposition plays a key role in cognitive dysfunction in people with mixed VCI.

The frequency of mixed AD-VCI pathology reported in this study corroborates with prevalence rates in previous autopsy and in-vivo studies. Among 87 autopsies of patients with dementia, 76 had AD. Of these 76, 44 (58%) had AD alone, and 32 (42%) had AD with CVD [132]. A community-based autopsy study found that 42 (45%) out of 94 cases of clinically diagnosed AD to also have significant cerebrovascular pathology [133]. An in-vivo study investigating the effect of cerebrovascular injury and amyloid plaques on cognitive dysfunction in clinically normal, cognitively impaired, and mildly demented participants found a significant amount of white matter hyperintensities in 14 (41%) out of 34 PiB-positive participants [134]. A similar study conducted by Lee and colleagues [109] investigating the rate of mixed vascular-AD pathology in people clinically diagnosed with subcortical vascular dementia found slightly lower rates of PiB positivity – 14 (31%) out of 45 patients exhibited both vascular and amyloid pathology. The lower rate of mixed pathology published by Lee and colleagues may be attributed to differences in the control sample. The average age of healthy controls in the Korean sample was 67.3 years compared with 55.3 years in our sample. Previous studies have found increased amyloid deposition with advancing age in healthy older adults [135,136]. Specifically, PiB retention increased with every decade, with 20%, 35% and 50% of the subjects presenting PiB-positive scans in the 61–70, 71–80, and 81+ year-old age groups respectively [136]. Additionally, the Tampere Autopsy Study comprising of 603 people found no individuals under the age of 50 to have moderate or frequent plaque deposition [137].
Thus, an older control sample may increase the threshold for PiB-positivity resulting in a lower percentage of PiB-positive individuals compared with a younger control sample. Based on rates published in this and other studies, almost half of the people with vascular lesions also have amyloid deposition.

Studies examining the relationship between amyloid plaques and cognitive function in AD have been equivocal. Some studies have reported an association between post-mortem plaque [138] load and in-vivo amyloid plaque deposition [105,139] with cognitive impairment. One post-mortem study found amyloid plaque in AD to be as strongly related to cognitive dysfunction as other established measures, including synapse loss, cell death, and tau hyperphosphorylation [138]. However, most large autopsy series have found the density of neocortical NFTs, and not amyloid plaques, to be better correlated with antemortem cognitive performance [140]. In-vivo studies are also inconclusive. A 2-year longitudinal PiB-PET study by Engel and colleagues [105] reported a significant negative correlation between MMSE score and PiB retention in the frontal, parietal, and occipital cortex at baseline. However, there were no significant correlations between PiB retention and MMSE score at 24-month follow-up, nor did change scores of PiB retention and MMSE show significant associations. This study also found episodic memory (as measured by the Rey Auditory Verbal Learning Task) to correlate with PiB retention in the frontal cortex, parietal cortex, posterior cingulum, and striatum at follow-up, however, change scores were not significant. A study by Edison and colleagues [141] investigating the role of amyloid deposition and hypometabolism with cognitive function found cortical PiB uptake to be correlated with impaired performance on recognition memory tests but this correlation was lost after withdrawing the AD subjects with normal baseline PiB uptake; yet, the correlation between cortical hypometabolism and memory function remained after the removal of an AD case with normal glucose metabolism. Thus, the authors suggested that amyloid deposition alone is unlikely to explain memory impairments and hypometabolism may be a stronger causative factor. However, data from a similar PET study by ADNI with a large sample of 426 individuals investigating the associations between amyloid, hypometabolism, and retrospective
longitudinal cognitive measurements suggests that the robust association between hypometabolism and cognitive function may be temporally based. Specifically, both hypometabolism and cognitive impairment become more pronounced with disease progression. The authors concluded that amyloid deposition has an early and subclinical impact on cognition that precedes and initiates metabolic changes [142].

Most autopsy and neuroimaging studies in AD have found no association between global amyloid burden and cognitive function [93,141,143,144]. As such, it is the general consensus that amyloid plaques do not correlate well with cognitive deficits in AD [145]. However, studies have also demonstrated that amyloid plaque deposition has reached a maximum in the clinical stages of AD and it may be that the relationship between amyloid plaques and memory has reached a plateau [146]. The Australian Imaging Biomarkers and Lifestyle research group reported that amyloid plaque deposition follows a sigmoidal curve over time: it takes 12 years for healthy controls with low PiB retention (SUVR=1.17) to reach abnormal/high levels of amyloid retention (SUVR=1.5), then it takes another 19 years to develop accumulation levels seen in AD (SUVR=2.33), at which time rates of Aβ deposition starts to slow, trending towards a plateau [147]. This is supported by several other PiB-PET studies that have found no substantial change in PiB retention over 1 [106], 2 [105,141], and up to 6-year periods [147]. To summarize, these studies indicate that amyloid accumulation begins in the preclinical phase and continues to accumulate in people with MCI and plateaus during clinical AD [106,145]. As such, the range of the data may be limited causing greater difficulty in detecting significant correlations. Notability, studies combining AD, MCI, and healthy control samples find PIB uptake to be correlated with cognitive function [148]. PiB-PET studies in MCI and healthy adults may better elucidate the impact of amyloid deposition on cognitive function.

People with MCI display a range of PiB retention, varying between levels seen in AD and healthy adults [149]. Multiple studies have indicated a detrimental effect of amyloid on cognitive function in people
with MCI [103,146,149,150]. PIB-positive subjects with MCI are significantly more likely to convert to AD than PIB-negative patients; furthermore, faster converters have higher PIB retention levels at baseline compared to slower converters [112]. In addition, both MCI participants and healthy adults who displayed PiB-positive scans performed worse on episodic memory measures compared with those who displayed PiB-negative scans. This study also reported a strong association between episodic memory and increased PiB binding in amnestic MCI and healthy adults; no differences were found for non-memory measures [146]. The strong association between PIB binding and conversion rates suggests that amyloid deposition is detrimental to cognitive function.

Our study did not find significant differences in cognitive function between PiB-positive and PiB-negative groups. However, there was a trend towards decreased global cognitive function as measured by the MOCA in the PiB-positive group compared with the PiB-negative group. In addition, we found PiB uptake to be significantly associated with the ADAS-Cog, a measure specifically developed to assess cognitive domains most affected by AD. Together age, gender, and education accounted for only 5.0% of the variance in ADAS-Cog performance. Adding PiB uptake to the regression model resulted in a significant R-square change of 36%. The total variance accounted by the final model was 41.0%. Further analyses of sub-scores within the ADAS-Cog revealed measures of memory and praxis to be significantly affected by amyloid deposition but not language. Our results concur and extend previous findings on the effect of amyloid in people with MCI and healthy adults. A study in people with MCI and healthy adults found increased PiB binding in neocortical regions to be significantly correlated with episodic memory impairment [146]. Two longitudinal studies in healthy adults found increased PiB retention to be associated with greater memory decline over time [136,151], specifically one study found verbal memory to be affected and not visual memory [151]. In addition to these studies, our study found amyloid deposition to also be associated with memory impairment in people with mixed VCI.
Our study also found increased amyloid to correlate with lower MOCA scores but not with MMSE scores. Based on standardized betas, PiB uptake ($\beta=-0.64$) was the strongest predictor of MOCA performance compared with age, gender, and education. To our knowledge, this is the first study to establish an association between PiB uptake and MOCA scores. These results corroborate with the neuropsychology literature in dementia. In comparing the MMSE with the MOCA, the MMSE has been shown to be insensitive to subtle declines in cognitive function, particularly to those of executive functions; a validation study involving 94 patients with MCI, found the MOCA showed 90% sensitivity for MCI, compared with 18% sensitivity with the MMSE [152]. Additionally, studies investigating PiB retention and MMSE scores have been inconclusive. A 2-year longitudinal study using PIB in patients with AD demonstrated a significant correlation between MMSE and PiB uptake in the frontal, parietal, and occipital cortex at baseline, however there were no significant correlations between MMSE and PiB retention at follow-up. Changes in MMSE score and PiB retention, expressed as percentages of baseline values, were also insignificant [105]. In addition, a cross sectional study by Rowe and colleagues [135] found that overall PiB uptake in the cortex was not related to MMSE scores in patients with mild to moderate AD. Data in patients with MCI and healthy adults have yielded similar results [135,148]. It has been established that the MOCA is a reliable measure in assessing MCI and our data suggests that the MOCA may also be a sensitive marker for AD pathology in VCI.

It is also not surprising that MOCA scores showed a significant association with AD pathology as executive dysfunctions have been reported in the early course of AD [153,154]. Older adults with AD displayed greater impairment on executive tasks that require concurrent manipulation of information [58] and attention [33] than older adults without AD. Specifically, it appears that divided attention and aspects of selective attention, such as set-shifting and response selection are particularly affected while sustained attention is preserved [33]. Furthermore, there is data in the literature indicating decreased performance on the Stroop test [155], Trail Making Test [58], and Digit Span Backwards test [156] in AD compared to
healthy age-matched adults. Our data, however, did not show a significant correlation between PiB retention with these specific measures of executive functions. Additionally, our data did not reveal a significant relationship between PiB retention and the EXIT-25. No other studies have reported data on the EXIT-25 and few studies have examined the effect of amyloid deposition on these specific executive processes. In a sample consisting of AD, MCI, and healthy participants, a linear regression found higher global PiB binding to be associated with the Trail Making Test parts A and B but no association was found for the Stroop and the Digit Span Forward and Backward tests after controlling for age and gender [148]. The Baltimore Longitudinal Study of Aging found declining performance on the Trails B over time to be significantly associated with higher PiB uptake in healthy older adults; however, this relationship was not significant with the inclusion of people with MCI [151]. Another study in healthy older adults found no difference between groups of high and low PiB uptake on either the Stroop Test, Trail Making Test (B Minus A), or the Digit Span Forward and Backward Test [157]. Although executive dysfunction has been reported in AD, it is unclear whether this is linked to amyloid pathology. It is possible that other variables associated with AD pathology such as brain atrophy, cerebral hypometabolism, and small vessel lesions are responsible for executive dysfunction.

Studies have reported reduced brain volume to be associated with lower cognitive function [158]. Woo and colleagues [159] found global atrophy to be associated with more severely impaired global cognition, working memory, mental speed, and executive functions. More specifically, data from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) study found lower executive function scores to be associated with advanced grey matter and cortical atrophy across broadly distributed regions, most notably in the bilateral parietal and temporal lobes in AD and MCI subjects [160]. These regions have also been implicated in studies using [(18)F] fluorodeoxyglucose positron emission tomography (FDG-PET); hypometabolism in frontal and parietotemporal regions are associated with decreased executive functions [161,162]. Furthermore, the finding on non-frontal regions in executive dysfunction was later confirmed
by ADNI data which also found hypometabolism in parietotemporal regions to be associated with executive dysfunction in AD and MCI patients [163]. Notably, frontal and parietotemporal regions have been shown to display increased PiB retention in AD [93]. Also, PiB positive subjects are much more likely to display hypometabolism compared to PiB negative subjects. Specifically, the parietal cortex shows an inverse relationship between amyloid deposition and glucose metabolism [164]. Increased whole brain and regional amyloid uptake, including frontal and parietal regions, are also positively correlated with whole brain atrophy [165]. Although it is unclear whether amyloid deposition is directly linked to executive functioning, these data show that there is likely an indirect effect and provides support for the hypothesis that cognitive function is modified by pathological processes initiated by amyloid accumulation [145].

The relationship between cerebrovascular disease and executive functions is well documented. As previously mentioned, a hallmark of vascular dementia is an impairment of executive functions. A systematic review and meta-analysis found white matter hyperintensities to increase dementia risk and to be specifically associated with faster decline in global cognitive performance, processing speed, and executive functions [166]. In addition, the Rotterdam Study of 1077 nondemented older adults found worse cognitive performance in people with severe white matter hyperintensities after accounting for the influences of age, gender, and education [27]. As our data included participants who were clinically diagnosed with VCI and had confirmed white matter pathology and executive dysfunction, we may have saturated our sample creating a “ceiling effect”. To expand, because all of our participants had executive dysfunction this may have limited the range of scores on the executive tests and therefore possibly obscuring the correlation and de-emphasizing the effect that amyloid may have on executive functions in mixed VCI. Another major limitation of this study is the small sample size. A small size increases Type II errors – due to the lack of statistical power – and may result in over-fitting. Conversely, due to the
multiple comparisons made in our analysis, we also increased the likelihood of Type I errors (i.e., inflated alpha).

Furthermore, it is unknown how white matter pathology may have uniquely contributed to performance on the ADAS-Cog and MOCA. It is unclear whether VCI pathology (i.e. lacunes and WMLs) could have fully accounted for the cognitive impairments or whether it may have an additive or synergistic effect in mixed VCI. This is particularly important as declines in memory performance, including those measured by the ADAS-Cog [167], have been linked to increased subcortical white matter disease [168]. A study published by Park and colleagues [31] investigating the relationship between CVD, PiB-PET, and cognitive function in subcortical VCI will help to elucidate these relationships. After adjusting for age, sex, hypertension, and APOE genotype, lacunes were found to be associated with memory and executive functions. White matter hyperintensities and PiB retention ratio were only associated with memory dysfunction. In addition, no positive correlations between cerebrovascular injury and amyloid burden were found and there were no interactive effects between CVD and amyloid pathology on cognitive performance. Park and colleagues suggested that CVD markers and amyloid burden act independently and not interactively to influence distinct cognitive domains.

Our current findings corroborate with these results. Our study also found amyloid uptake to be associated with memory impairments and the data by Park and colleagues give confidence that our effects were not confounded by CVD. However, we found executive functions, as measured by the MOCA, to be associated with PiB retention. Although the MOCA does include a memory subtest, it places greater emphasis on tasks of executive functioning [169]. The MOCA test includes a composite score of multiple aspects of executive functions including an alternation task (adapted Trail Making B), a phonemic fluency task, a two-item verbal abstraction task, a sustained attention task (target detection with tapping), concentration task (serial subtraction task), and a working memory task (Digits Forward and Backward).
In addition, visuospatial abilities are assessed using a clock-drawing task and a three-dimensional cube copy task. The executive component of the Park study only included the phonemic and semantic Controlled Oral Word Association Test and the Stroop Test. A complex statistical study conducted by ADNI found a composite score (ADNI-EF) of Category Fluency, Clock Drawing, WAIS-R Digit Symbol, Digit Span Backwards, and the Trail Making Test including Trails A, Trails B, and Trails B minus Trails A to be superior to any independent measure of executive functioning. Specifically, ADNI-EF was sensitive to capturing changes in cognitive function over time, it was the strongest baseline predictor of conversion to AD, and it was the only predictor associated with white matter hyperintensities [170]. The MOCA is much more similar to the ADNI-EF compared to the composite score created by Park and colleagues. It is important to recognize that executive functions involve multiple cognitive processes and different tests will demand different neurological responses. As such, the non-significant interaction between specific executive processes with WMLs and PiB retention may be limited by the power of the executive test to detect an effect – tests that measure multiple cognitive processes (i.e. MOCA and ADAS-Cog) as opposed to tests that measure specific processes (i.e. Trail Making Test, Verbal Digit Span Forward and Backward Test, and Stroop Test) may be more reliable at detecting changes in cognition associated with vascular and amyloid pathology. We propose that amyloid deposition plays a significant role in both memory and executive impairments in VCI.
5. Conclusion, Limitations, and Future Directions

As Canada’s population ages, dementia will become a significant health care burden. Alzheimer’s disease and VCI are the two most common forms of dementia [18]. Traditionally, these two types of dementias were considered to be distinct and unrelated. However, we now know that AD and VCI can and do co-exist. Research suggests that there in an interactive relationship between the primary mechanisms of pathogenesis. Specifically, amyloid negatively impacts cerebrovascular function and cerebral ischemia is thought to be a powerful modulator in amyloidosis [63]. Furthermore, multiple large autopsy series have found a high prevalence of co-existing VCI and AD pathology [10]. Yet, the guidelines for the clinical diagnosis of VCI has not recognized these interactions and mixed VCI may be clinically underdiagnosed as a cause of cognitive impairment. Thus, the purpose of this study was to determine the frequency of mixed VCI in patients clinically diagnosed with VCI. In addition, we investigated how co-existing amyloid pathology may affect cognitive function in people with VCI.

Using PiB-PET imaging, our study found 44% of people clinically diagnosed with VCI had abnormal levels of amyloid deposition and were categorized to have mixed VCI. Importantly, increased PiB retention was associated with increased cognitive dysfunction, specifically in the domains of memory and executive functioning as measured by the ADAS-Cog and MOCA. However, the ascertainment of these conclusions was not without limitations. First, our study was limited by our small sample size, particularly in the control group. It is important to note that the frequency of PiB-positivity is reliant on the threshold determined by the control population. As such, differences in control samples will result in differences in the frequency of mixed VCI. Although the frequency of mixed VCI here are in agreement with other published reports [132-134], it may be more informative for future studies to use a young control group (providing a prudent threshold), an age-matched control group, and an AD comparison group. These analyses may better speak to the association between varying levels of amyloid deposition and the magnitude of cognitive dysfunction.
As another limitation, our study did not account for the effect of NFT on cognitive function. This is of particular importance as both amyloid and NFT are considered pathological hallmarks of AD. Although amyloid plaques play a key role in AD pathogenesis, post-mortem studies have revealed that neocortical NFT burden showed a stronger correlation with dementia severity [140] and NFT seems to mediate the association of amyloid on cognitive function [171]. A study using \(^{18}\)F-FDDNP, which binds to both Aβ and NFT, found \(^{18}\)F-FDDNP binding to be associated with impairments of episodic memory [148], however, the role of tau pathology alone on cognitive dysfunction remains unclear. Currently there are no tracers available to image tau pathology alone in humans in-vivo. Three recent publications describe initial attempts to identify tau binding PET ligands, including F-labelled T807 and T808 [172], F-labelled THK compounds [173] and \(^{11}\)C-PBB3 [174]. These ligands show promising potential, however more work is required before in-vivo imaging on tau pathologies in humans is possible [175]. The development of tau-selective radioligands will provide future studies an opportunity to elucidate the interrelations between Aβ, NFT, and CVD.

Although PET imaging has provided unique opportunities to image AD pathology in-vivo, it is not without methodological limitations. One limitation includes partial volume effects (PVEs), which degrade the quantitative accuracy of an image. Specifically, partial volume effects in PET imaging refer to two phenomena: the blurring of an image due to the finite spatial resolution of the scanner and the sample voxel size of an image. The blurring causes activity to spill out into neighboring regions and activity from surrounding regions to spill in. Counts are not lost due to PVEs; rather, they are displaced from their true location. The second effect is induced by voxel size and is referred to as the tissue-fraction effect. Each voxel is likely to contain multiple tissue types and different tissue types within a voxel contributes differently to tracer concentration. The value extracted from each voxel is the average activity from various tissue types. Thus, the mean activity of each voxel does not accurately describe the true tracer distribution. Both effects cause errors in image quantification. The degree to which an object is affect by
PVEs is dependent on size, with greater PVEs on smaller objects [176]. As such, partial volume corrections are important in samples of people with neurodegenerative diseases characterized by brain atrophy [177]. Both AD and vascular dementia are characterized by cerebral atrophy and this may cause overestimation of amyloid deposition. Although partial volume corrections are more critical in longitudinal studies of amyloid deposition (to correct for the effect of brain atrophy over time), the correction of PVEs would generally improve the regional quantification of amyloid deposition [176].

In addition to methodological limitations coupled with imaging pathology, we should also be cognizant of environmental confounders. Of specific importance is the notion of cognitive reserve. The hypothetical construct of cognitive reserve has been proposed as a moderator between pathology and clinical outcome, thus accounting for the individual difference in susceptibility to age-related brain changes and pathology [178]. This concept is particularly relevant in AD, as research has often demonstrated a lack of linearity between pathology and clinical symptoms [179]. For example, several studies have identified heavy amyloid burden in a significant number of individuals who do not display clinical evidence of AD. Autopsy and PET studies have determined the prevalence of amyloid burden in cognitively normal elderly persons to be between 10-30% [180-183]. The cognitive reserve hypothesis purports that factors such as education, occupation, environmental richness, exercise, and lifestyle in general may influence the manifestation of pathophysiology [184]. Indeed, several studies have indicated that people with higher levels of education are more resilient to memory declines and can better cope with AD brain pathology [185-187]. Data has also suggested that engagement in leisure activities (knitting, reading, music, walking, visiting friends or relatives, being visited by friends or relatives, physical conditioning, community outings, volunteer work, etc.) may reduce the risk of incident dementia by providing a reserve that delays the onset of clinical symptoms [188]. Furthermore, animal models suggest that environmental richness might act directly to prevent or slow the accumulation of AD pathology [189]. There is strong evidence supporting the role of cognitive reserve within AD research. Future studies should not only
control for these variables, but develop a model of disease progression that integrates the complex interrelations between pathology and environmental influences for a more complete understanding of the evolution of dementia.

Although the rates of dementia are projected to skyrocket over the next several decades, research suggests that rates of dementia may be decreasing through the control of vascular risk factors [190]. Our study found that almost half of the patients clinically diagnosed with VCI actually have co-existing AD pathology. As such, people with mixed VCI represent an ideal target population as CVD risk factors can be controlled and modified. Furthermore, we have shown that increased amyloid accumulation in VCI is associated with memory and executive dysfunctions. Thus, future clinical trials in mixed VCI may need to focus on amyloid accumulation to preserve cognitive functioning in people with mixed VCI. The preservation of their cognitive and functional capabilities will likely maintain and prolong their ability to live independently. Delaying the onset and progression of dementia by only one year would reduce 9.2 million cases of dementia by 2050 in the US alone [191].
## Tables

Table 1. Descriptive statistics for variables of interest

<table>
<thead>
<tr>
<th>Variables</th>
<th>PiB+ (n=8)</th>
<th>PiB- (n=10)</th>
<th>Group Differences</th>
<th>Total VCI (N=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>74.9 (6.0)</td>
<td>73.5 (6.9)</td>
<td>t(16)= -0.48, p=0.66</td>
<td>74.1 (6.3)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.3 (1.6)</td>
<td>27.4 (2.3)</td>
<td>t(16)= 0.38, p=0.38</td>
<td>27.8 (2.0)</td>
</tr>
<tr>
<td>Female Sex (%)</td>
<td>5 (63)</td>
<td>3 (30)</td>
<td>x²(1)= 1.90, p=0.17</td>
<td>8 (44)</td>
</tr>
<tr>
<td>FCI</td>
<td>3.8 (1.5)</td>
<td>3.7 (2.1)</td>
<td>t(16)= -0.06, p=0.94</td>
<td>3.7 (1.8)</td>
</tr>
<tr>
<td>Education, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school education</td>
<td>2 (25)</td>
<td>3 (30)</td>
<td></td>
<td>5 (27.7)</td>
</tr>
<tr>
<td>Trade or professional certificate or diploma</td>
<td>4 (50)</td>
<td>2 (20)</td>
<td>x²(3)= 1.61, p=0.66</td>
<td>6 (33.3)</td>
</tr>
<tr>
<td>University education</td>
<td>2 (25)</td>
<td>5 (50)</td>
<td></td>
<td>7 (38.9)</td>
</tr>
<tr>
<td>Cognitive Assessments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOCA</td>
<td>22.63 (2.88)</td>
<td>24.70 (1.64)</td>
<td>t(16)= -1.93, p=0.07</td>
<td>23.8 (2.4)</td>
</tr>
<tr>
<td>ADAS-Cog</td>
<td>11.18 (6.40)</td>
<td>8.91 (1.44)</td>
<td>^t(16)= -1.93, p=0.07</td>
<td>9.9 (4.2)</td>
</tr>
<tr>
<td>Exit 25</td>
<td>9.50 (5.18)</td>
<td>10.40 (3.37)</td>
<td>t(16)= -0.45, p=0.66</td>
<td>10.0 (4.1)</td>
</tr>
<tr>
<td>Stroop CW-W</td>
<td>54.37 (21.79)</td>
<td>66.85 (29.67)</td>
<td>t(16)= -0.99, p=0.34</td>
<td>61.3 (26.5)</td>
</tr>
<tr>
<td>Trails B-A</td>
<td>52.86 (37.19)</td>
<td>52.74 (14.33)</td>
<td>^t(16)= 0.01, p=0.99</td>
<td>52.8 (26.0)</td>
</tr>
<tr>
<td>Digits B-F</td>
<td>2.75 (2.43)</td>
<td>3.00 (3.37)</td>
<td>t(16)= -0.18, p=0.86</td>
<td>2.8 (2.9)</td>
</tr>
<tr>
<td>PiB uptake</td>
<td>0.27 (2.8)</td>
<td>-0.08 (0.05)</td>
<td>t(16)= 5.01, p=0.00</td>
<td>0.07 (0.23)</td>
</tr>
</tbody>
</table>

MMSE = Mini Mental State Examination; MOCA = Montreal Cognitive Assessment; Stroop = Stroop colour words condition subtracted by Stroop coloured x’s condition; Trails = Trail B subtracted by Trail A; Digits = Digits Forward subtracted by Digits Backward.

^ = Levene’s test for homogeneity of variance was violated. A t-test removing outliers was conducted and this did not change the results.
Table 2. Pearson correlation results

<table>
<thead>
<tr>
<th>Controlling for Age, Gender, Education</th>
<th>Global Uptake</th>
<th>MOCA</th>
<th>ADAS Cog</th>
<th>EXIT-25</th>
<th>Stroop</th>
<th>Trails</th>
<th>Digits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global Uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOCA</td>
<td>-0.63*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADAS-Cog</td>
<td>0.61*</td>
<td>-0.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EXIT-25</td>
<td>0.16</td>
<td>-0.28</td>
<td>0.64*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroop</td>
<td>0.11</td>
<td>-0.17</td>
<td>0.27</td>
<td>0.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trails</td>
<td>0.21</td>
<td>-0.20</td>
<td>0.21</td>
<td>0.36</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digits</td>
<td>-0.22</td>
<td>0.43</td>
<td>-0.43</td>
<td>-0.31</td>
<td>0.10</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

*=significant at p<0.05

Table 3. Multiple linear regression model assessing the contribution of PiB uptake to MOCA

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>r</th>
<th>R²</th>
<th>R² Change</th>
<th>Unstandardized B (Standard Error)</th>
<th>Standardized β</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td>0.13</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.09</td>
<td></td>
<td></td>
<td>-0.03 (0.11)</td>
<td>-0.08</td>
<td>0.78</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.01</td>
<td></td>
<td></td>
<td>-0.21 (1.32)</td>
<td>-0.04</td>
<td>0.87</td>
</tr>
<tr>
<td>Education</td>
<td>-0.11</td>
<td></td>
<td></td>
<td>-0.26 (0.73)</td>
<td>-0.10</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td>0.64</td>
<td>0.41</td>
<td>0.39*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.09</td>
<td></td>
<td></td>
<td>0.00 (0.09)</td>
<td>0.06</td>
<td>0.98</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.01</td>
<td></td>
<td></td>
<td>0.53 (1.09)</td>
<td>0.11</td>
<td>0.63</td>
</tr>
<tr>
<td>Education</td>
<td>-0.11</td>
<td></td>
<td></td>
<td>-0.24 (0.59)</td>
<td>-0.09</td>
<td>0.69</td>
</tr>
<tr>
<td>PiB-uptake</td>
<td>-0.62*</td>
<td></td>
<td></td>
<td>-6.85 (2.34)</td>
<td>-0.64</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*=significant at p<0.01
Table 4. Multiple linear regression model assessing the contribution of PiB uptake to ADAS-Cog

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>r</th>
<th>$R^2$</th>
<th>$R^2$ Change</th>
<th>Unstandardized B (Standard Error)</th>
<th>Standardized β</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.18</td>
<td>0.05</td>
<td>0.05</td>
<td>0.09 (0.18)</td>
<td>0.13</td>
<td>0.84</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.16</td>
<td></td>
<td></td>
<td>-0.94 (2.22)</td>
<td>-0.12</td>
<td>0.64</td>
</tr>
<tr>
<td>Education</td>
<td>0.12</td>
<td></td>
<td></td>
<td>0.37 (1.22)</td>
<td>0.08</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td>0.64</td>
<td>0.41</td>
<td>0.36*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.18</td>
<td></td>
<td></td>
<td>0.03 (0.15)</td>
<td>0.05</td>
<td>0.83</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.16</td>
<td></td>
<td></td>
<td>-2.16 (1.9)</td>
<td>-0.26</td>
<td>0.27</td>
</tr>
<tr>
<td>Education</td>
<td>0.12</td>
<td></td>
<td></td>
<td>0.34 (1.00)</td>
<td>0.07</td>
<td>0.74</td>
</tr>
<tr>
<td>PiB-uptake</td>
<td>0.57*</td>
<td></td>
<td></td>
<td>11.20 (4.00)</td>
<td>0.62</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*=significant at p<0.05
Figures

Figure 1. Scatterplot of global PiB uptake
Figure 2. Scatterplot of PiB uptake and MOCA scores

Scatterplot of raw scores with line of best fit.
Figure 3. Scatterplot of PiB uptake and ADAS-Cog scores

Scatterplot of raw scores with line of best fit.
References


