Quantification of diverse cerebrovascular pathologies on MRI in older individuals and their relationships to cognition in a multimodal MRI index.

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Quantification of diverse cerebrovascular pathologies on MRI in older individuals and their relationships to cognition in a multimodal MRI index.

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A thesis in fulfilment of the requirements of Doctor of Philosophy

UNSW Medicine. School of Psychiatry.

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Abstract

Cerebrovascular disease (CVD) is a leading cause of morbidity and mortality, with manifold clinical consequences including vascular dementia (VaD), the second most common dementia subtype. CVD also worsens the risk and expression of Alzheimer's disease (AD). Diagnosing VaD requires determining whether a patient's cognitive deficits can be explained by the current CVD burden. However, CVD is markedly pleomorphic and its full extent has been difficult to determine. Traditional markers of CVD such as white matter hyperintensities (WMH) are inconsistently associated with clinical outcomes. There is an unmet need to better quantify total CVD burden and relate it to cognition and dementia.

CVD indices have been published, which combine information from several neuroimaging markers, in the hope to better capture the variability seen on neuroimaging and lead to more robust associations with cognition.

In this thesis, the existing literature on neuroimaging and neuropathological indices of CVD was systematically reviewed. The contributions of two common but under-researched MRI CVD markers, cerebral microbleeds (CMB) and dilated perivascular spaces (PVS), were then assessed. The second chapter examines the association of CMB with both cross sectional and longitudinal impairment, finding associations with executive function and visuospatial function respectively. There was no existing reliable rating scale for quantification of PVS and the development of this is the theme of the third chapter. The fourth chapter examines the associations of dilated PVS with longitudinal cognitive impairment and incident dementia. I found that individuals with the most severe PVS had declines in Global Cognition and their presence triples the risk of developing dementia over 8 years.

The fifth chapter describes the development of an MRI-based composite CVD index and its validation in two independent cohorts. Peak skeletonised mean diffusivity, a DTI measure, and WMH volume contributed most to the variability seen in Global Cognition. The Index explained 9% of the proportion of the variance seen in the

development sample and 5 and 13 % in the validation cohorts respectively. It performed better than the most widely used CVD index in all cohorts examined.

This work broadens the range of vascular pathologies used in the determination of the total CVD burden. The resulting composite index, while needing further refinement, represents a promising step-forward in the assessment of such burden in an individual, which could potentially help clinicians and researchers in determining the vascular contributions to dementia.

Thesis Title and Abstract sheet

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Thesis Abstract

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Candidate's Declaration

I confirm that where I have used a publication in lieu of a chapter, the listed publication(s) above meet(s) the requirements to be included in the thesis. I also declare that I have complied with the Thesis Examination Procedure.

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Abbreviations

AD	Alzheimer's disease
AC	anterior commissure
ADDTC	Alzheimer's Disease Diagnostic and Treatment Centers
Adj R^2	adjusted R^2
ApoE	apolipoprotein E
ASL	arterial spin labelling
B	unstandardized regression coefficient
BBB	blood brain barrier
BG	basal ganglia
BMI	body mass index
CAA	cerebral amyloid angiopathy
CAA	cerebral amyloid angiopathy
CAMDEX	Cambridge Mental Disorders of the Elderly Examination
CBF	cerebral blood flow
CDR	Clinical Dementia Rating scale
CI	confidence internal
CMB	cerebral microbleed
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
CSF	cerebrospinal fluid
CSO	centrum semiovale
CT	computerised tomography
CVD	cerebrovascular disease
CVDPS	cerebrovascular parenchymal pathology scores.
DLB	dementia with Lewy bodies
DSM	Diagnostic and statistical manual of mental disorders
DTI	diffusion tensor imaging

DV	dependent variable
DWI	diffusion weighted imaging
FA	fractional anisotropy
FAS	Controlled Oral Word Association Test
FLAIR	fluid-attenuated inversion recovery
FSL	FMRIB Software Library
GRE	gradient-recalled echo.
HS	hippocampal sclerosis
ICC	intraclass correlation coefficient
ICD	International Classification of Diseases
ICV	intracranial volume
IQR	interquartile range
LL	lower limit
LMM	linear mixed-models
LVD	large vessel disease
MAS	Sydney Memory and Ageing Study
MCI	mild cognitive impairment
MD	mean diffusivity
MID	multi-infarct dementia
MMSE	mini-mental state examination
MRI	magnetic resonance imaging
MTA	medial-temporal atrophy
NC	normal controls
NINDS-AIREN	National Institute of Neurological Disorders and Stroke Association Internationale Pour la Recherche et l'Enseignement en Neurosciences.
NMDA	N-methyl-D-aspartate
OATS	Older Australian Twin Study
PET	positron emission tomography
ppts	participants

PPV	positive predictive value
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSD	post-stroke dementia
PSMD	peak skeletonised mean diffusivity
PVS	perivascular spaces
R2	coefficient of determination / proportion of variance
RAVLT	Rey Auditory Verbal Learning Test
RCCS	Renji Cerebral SVD Cohort Study
ROC	receiver operating characteristic
SD	standard deviation
SE	standard error
SLE	systemic lupus erythematosus
SPECT	single-photon emission computerized tomography
STRIVE	STandards for ReportIng Vascular changes on nEuroimaging
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
SVD	small vessel disease
SWI	susceptibility weighted imaging
TE	echo time
TI	inversion time
TIA	transient ischemic attack
TMT	Trail Making Test
TR	repetition time
UL	upper limit
UQ	upper quartile
VaD	vascular dementia
VASCOG	International Society for Vascular Behavioral and Cognitive Disorders
VCD	vascular cognitive disorders
VCI	vascular cognitive impairment
VCING	vascular cognitive impairment neuropathology guidelines

VICCS   Vascular Impairment of Cognition Classification Consensus Study

WMH   white matter hyperintensity

β   standardised coefficient

Chapter 1: Introduction

1.1 Organisation of the thesis

Chapter 1: Introduction provides a rationale and background for the thesis, namely explaining why the accurate quantification of CVD burden is an important goal and the limitations of our existing methods. It includes the published review of Vascular cognitive disorder[1], which discusses the subject at a broad level. The potential benefits of a multi-modal CVD index over single measures are discussed, including in the published systematic review entitled "Neuroimaging and neuropathology indices of cerebrovascular disease burden: A systematic review"[2]. The introduction finishes with the aims and hypotheses of the Thesis.

Chapter 2: The relationship of cerebral microbleeds (CMB) to cognition and incident dementia in non-demented older individuals explores the contribution of CMB to both cross-sectional and longitudinal cognitive impairment as well as incident dementia over four years of follow up.

Chapter 3: Development and validation of a rating scale for dilated perivascular spaces (PVS) on MRI details the development of a novel PVS rating scale. This was necessary as any exploration of the contribution of PVS was hampered by the poor reliability of existing rating scales.

Chapter 4: The association of PVS with longitudinal cognitive decline and incident dementia examines the relationship of PVS, quantified with the aforementioned rating scale, with cognitive impairment and incident dementia. By this time, follow-up data for up to eight years were available.

Chapter 5: Development and validation of a novel MRI index to quantify the relationship of cerebrovascular disease with cognition uses the information derived from early chapters to construct and then validate a multi-modal CVD index. This includes examination of the contribution of both CMB and PVS. The primary and secondary hypotheses stated in the introduction are tested.

Finally, *Chapter 6* provides a brief summary of the four data-based chapters and integrates the significant findings of the Thesis. It discusses the limitations and challenges seen in the field of CVD quantification and the Index more specifically. It concludes by articulating the novel contribution the Thesis has made to knowledge in neuroimaging and vascular cognitive impairment, how best clinical knowledge translation may be achieved and suggests future research directions.

The five published manuscripts emanating from the Thesis are attached in the Appendix as .pdf files. Please note that continuous numbering of references has been used in the Thesis, so citation numbers will differ from the published manuscripts.

1.2   The importance of cerebrovascular disease.

The term cerebrovascular disease (CVD) has been used primarily in three ways: to designate damage to cerebral blood vessels themselves; when discussing parenchymal damage as a consequence of vascular pathology; and finally, to refer to disorders resulting from, or associated with, the vessel or tissue pathology.

A common method of CVD subdivision is into large vessel disease (LVD) and small vessel disease (SVD). Large vessels are the arteries and veins than can be visualised macroscopically and damage from these can produce a variety of clinical phenotypes, the most serious of which include ischaemic or haemorrhagic strokes. Certain types of vascular cognitive disorders may also result[1].

SVD refers to damage to small arteries, arterioles, venules, and capillaries of the brain. SVD can results from multiple pathologies – see Table 1.1 and can have manifold clinical consequences, including lacunar stroke, vascular cognitive disorders, gait disturbance, urinary incontinence and neuropsychiatric symptoms.

Table 1.1: Aetiopathogenic classification of cerebral small vessel diseases

Classification	Comments
Type 1: arteriolosclerosis (or age-related and vascular risk-factor-related small vessel diseases)	Pathologies include: fibrinoid necrosis, lipohyalinosis, microatheroma microaneurysms (saccular, lipohyalinotic, asymmetric fusiform, bleeding globe), segmental arterial disorganisation
Type 2: sporadic and hereditary cerebral amyloid angiopathy	
Type 3: inherited or genetic small vessel diseases distinct from cerebral amyloid angiopathy	E.g., CADASIL=cerebral autosomal dominant arteriopathy with subcortical ischaemic strokes and leukoencephalopathy.
Type 4: inflammatory and immunologically mediated small vessel diseases	E.g., Churg-Strauss syndrome and various vasculitides associated with connective tissue disorders including systemic lupus erythematosus.
Type 5: venous collagenosis	
Type 6: other small vessel diseases	E.g., post-radiation angiopathy.

Table adapted from Pantoni et al.[3]

It is worth noting that the LVD/SVD subdivision is non-exclusive and it is very common for older individuals with large vessel disease to also have neuroimaging evidence of some degree of SVD[1]. This is partly because CVD is extremely common in the elderly. On post-mortem examination, 50% to 84% of the brains of people who die aged 80 or older show appreciable cerebrovascular lesions[4].

CVD is responsible for high levels of morbidity and mortality, and was the third commonest cause of death amongst Australians in 2018 with "Dementia including Alzheimer's" rated second[5]. As will be discussed, the latter category itself has a large contribution from vascular pathology. In terms of worldwide morbidity, among non-communicable diseases, stroke is the second largest contributor to disability[6].

Chapter 1.3: Vascular Cognitive Disorder[1], explores the concept of vascular cognitive disorders and the complex relationship between CVD pathology, cognitive impairment and dementia.

In addition to the direct consequence of CVD leading to vascular dementia (VaD) and vascular cognitive impairment (VCI), there is increasing evidence that vascular risk factors and CVD are involved in the pathogenesis of Alzheimer's disease (AD)[7-9]. CVD and AD share common risk factors such as midlife hypertension, diabetes, Apolipoprotein ε4 (APOE-ε4) isoform, hypercholesteremia and age. Clinically, CVD and the vascular risk factors associated with it worsen the risk and expression of AD. Midlife hypertension, midlife obesity and late life diabetes are responsible for 5, 2 and 3% of the dementia population attributable fraction respectively[10]. White matter hyperintensity (WMH) severity increases the risk of incident dementia[11] and AD more specifically[7, 12]. Although results have been mixed[13, 14], WMH severity has been associated with accelerated cognitive decline in established dementia[15].

With the failure to develop disease modifying therapies for dementia, there has been an increased focus on the modification of vascular risk factors, with up to 35% of the population attributable fraction potentially preventable over the life course[10]. Indeed, there is now evidence that dementia incidence in developed countries has started to decrease, in association with improvement in certain vascular risk factors[16, 17] (although obesity and diabetes have increased over time).

In summary, CVD is extremely common and causes a range of deleterious consequences both by itself and by interaction with other pathologies. Importantly, CVD may be preventable and addressing vascular risk factors, may be the most effective strategy for reducing dementia rates.

1.3 Publication: Paradise MB, Sachdev PS. Vascular Cognitive Disorder. Semin Neurol 2019;39:241-250.

Vascular Cognitive Disorder

Abstract

The term vascular cognitive disorder (VCD) refers to a heterogeneous group of disorders in which the primary feature is cognitive impairment attributable to cerebrovascular disease (CVD). This includes not only vascular dementia (VaD) but also cognitive impairment of insufficient severity to meet diagnostic criteria for dementia. VCD is recognized as the second most common cause of dementia after AD, but prevalence rates vary widely according to the diagnostic criteria employed. There have been recent attempts to standardize diagnostic criteria. VCD incorporates a range of neuropathological mechanisms including post-stroke impairment, small and large vessel disease and cases of mixed-pathology, with CVD interacting with Alzheimer's disease (AD) and other neuropathologies. Recent neuroimaging data have improved our understanding of the etiology of VCD. Symptomatic treatments for VaD have modest benefit and there is increased focus on the primary and secondary preventative benefits of vascular risk factor control.

Introduction and diagnostic criteria

The long-standing view of dementia of vascular etiology being caused by "narrowing of the arteries" was challenged in the 1970s with the concept of multi-infarct dementia[18], whereby vascular dementia was considered to be due to the cumulative effect of multiple small and/or large brain infarcts. This in turn, was seen as too narrow a concept, with the recognition that multiple pathologies may result in vascular cognitive disorder (VCD) – not only multiple cortical and/or subcortical infarcts, but also strategic single infarcts, non-infarction white matter lesions, hemorrhages, and hypoperfusion[19]. VCD is therefore an umbrella term for a heterogeneous group of disorders in which the primary feature is cognitive impairment attributable to cerebrovascular disease (CVD). These pathologies may also be referred by the plural term vascular cognitive *disorders*, reflecting the multiplicity of pathologies and diseases involved. VCD subsumes the term vascular cognitive impairment (VCI), which has been used synonymously with VCD but more frequently as mild cognitive impairment[20] of vascular origin. Individuals with VCI would typically not meet the dementia requirements of most diagnostic criteria due to the severity of their cognitive impairment, the cognitive domains affected or the lack of functional impairment. The importance of the milder forms of VCD is seen in their role in prevention and in etiological research.

Diagnostic criteria for VCD[21] have evolved to reflect our improved understanding of the disorders, but differ considerably in their requirements of focal neurological signs, cognitive impairment (they do not include mild VCD which may account for half of those with VCD[22]) and relevant CVD neuroimaging, and produce very different vascular dementia (VaD) prevalence rates[21]. Due to this heterogeneity, the International Society for Vascular Behavioral and Cognitive Disorders (VASCOG) produced criteria for VCD[23] in 2014 which were harmonized with the DSM-5 criteria[24]. These latter two criteria recognize that VCD is multifactorial, its presentation is different from that of AD, cognitive and functional impairment lie on a continuum from normal to mild disorder to a dementia (or major neurocognitive disorder in DSM-V), and neuroimaging increases the certainty of diagnosis. A summary of the VASCOG criteria is presented in Table 1.2.

Table 1.2: VASCOG criteria for Vascular Mild Cognitive Disorder and Vascular Dementia

PRESENCE OF A COGNITIVE DISORDER

Mild Cognitive Disorder

A. Acquired decline in one or more cognitive domains, as evidenced by the following:
   1. Concerns of a patient, knowledgeable informant, or clinician of mild levels of decline from a previous level of cognitive functioning; *and*
   2. Evidence of modest deficits on objective cognitive assessment based on a validated measure of neurocognitive function

B. The cognitive deficits are not sufficient to interfere with independence (i.e., instrumental activities of daily living are preserved), but greater effort, compensatory strategies, or accommodation may be required to maintain independence.

Dementia (or Vascular Major Cognitive Disorder)

A. Evidence of substantial cognitive decline from a documented or inferred previous level of performance in one or more of the domains outlined above. Evidence for decline is based on the following:
   1. Concerns of the patient, knowledgeable informant, or clinician of significant decline in specific abilities *and*
   2. Clear and significant deficits in objective assessment based on a validated objective measure of neurocognitive function

B. The cognitive deficits are sufficient to interfere with independence.

EVIDENCE FOR PREDOMINANTLY VASCULAR CAUSE OF COGNITIVE IMPAIRMENT

A. One of the following clinical features:
   1. The onset of the cognitive deficits is temporally related to one or more cerebrovascular events (CVEs). The evidence of CVEs is one of the following:
      a. Documented history of a stroke, with cognitive decline temporally associated with the event
      b. Physical signs consistent with stroke
   2. Evidence for decline is prominent in speed of information processing, complex attention and/or frontal executive
      functioning in the absence of history of a stroke or transient ischemic attack. One of the following features is also present:
      a. Early presence of a gait disturbance (small step gait [*marche petits pas*] or magnetic, apraxic-ataxic, or parkinsonian gait); This may also manifest as unsteadiness and frequent, unprovoked falls.
      b. Early urinary frequency, urgency, and other urinary symptoms not explained by urologic disease
      c. Personality and mood changes—abulia, depression, or emotional incontinence.

B. Presence of significant neuroimaging (MRI or CT) evidence of cerebrovascular disease (CVD)

EXCLUSION CRITERIA

A. History
   1. Early onset of memory deficit and progressive worsening of memory and other cognitive functions such as language (transcortical sensory aphasia), motor skills (apraxia), and perception (agnosia) in the absence of corresponding focal lesions on brain imaging or history of vascular events
   2. Early and prominent parkinsonian features suggestive of Lewy body disease
   3. History strongly suggestive of another primary neurologic disorder

B. Neuroimaging
   1. Absent or minimal cerebrovascular lesions on CT or MRI
   2. Other medical disorders severe enough to account for memory and related symptoms:

      a. Other disease of sufficient severity to cause cognitive impairment (e.g., brain tumor, multiple sclerosis, encephalitis)

      b. Major depression, with a temporal association between cognitive, toxic, and metabolic abnormalities

3. For research—presence of biomarkers for Alzheimer disease (AD; cerebrospinal Aβ and pTau levels or amyloid imaging at accepted thresholds) exclude diagnosis of probable VCD, and indicate AD with CVD.

CT, Computed tomography; MRI, magnetic resonance imaging; VCD, vascular cognitive disorder.

Epidemiology

Prevalence and incidence rates for VCD vary widely, depending on the diagnostic criteria applied and the populations studied [25]. VaD is the second most common dementia after AD, with prevalence estimates of around 16% of all dementia cases[26], with the age-standardized prevalence of VaD estimated to be 1.6% compared to 4.4% for AD in a European population aged over 65 years. Prevalence doubles every 5 years[26].

Post-stroke dementia (PSD) is common - 3.5 to 5.6 fold more frequent in patients compared to stroke-free controls[27] - with 6 to 32% dementia prevalence three months after stroke [28]. The prevalence of post-stroke cognitive impairment not meeting diagnostic criteria for dementia, is high but very variable - around 25 to 60% [29] - and this is a source of ongoing interest[28].

Vascular lesions are common in autopsy studies of dementia cases, with up to 75% of cases having some evidence of vascular pathology[30] and approximately one third showing significant vascular pathology[31]. A review of neuropathologic studies has shown a wide range in prevalence of VaD, from 0.03% to 85.2%, with a median figure of about 11%.[31]

Mechanisms and pathophysiology

The clinical manifestations and causes of VCD are very heterogeneous, reflecting the diversity of parenchymal lesions and vascular pathology seen in CVD. One approach to the pathophysiology of VCD has been to examine the contributions made by large vessel disease, small vessel disease (SVD), non-infarct ischemic changes, hemorrhages and other factors, as summarized in Table 1.3.

Table 1.3: Parenchymal lesions of Vascular Cause Associated with Vascular Cognitive Disease

Large vessel disease	Multiple infarcts
	Single strategically placed infarct
Small vessel disease	Multiple lacunar infarcts in white matter and deep gray matter nuclei
	Ischemic white matter change
	Dilation of perivascular spaces
	Cortical microinfarcts
	Cortical and subcortical microbleeds
Hemorrhage	Intracerebral hemorrhage
	Multiple cortical and subcortical microbleeds
	Subarachnoid hemorrhage
Hypoperfusion	Hippocampal sclerosis
	Laminar cortical sclerosis

Large Vessel Disease

The likelihood of a single or multiple lesions leading to VaD is increased by the presence of multiple infarcts[18], larger infarcts or cortical infarcts[32] particularly those in the left hemisphere, or anterior and posterior cerebral artery lesions.[33] Solitary, "strategic" lesions can lead to VCD, particularly if they occur in specific brain regions and can result in characteristic PSD cognitive syndromes[34].

Atherosclerosis in large extracranial or intracranial vessels can cause ischemia either through a reduction of blood flow (hemodynamic cause) or due to intra-artery embolism. Severe carotid stenosis is common but large artery atherosclerosis accounts for only 30% of strokes, compared to cardioembolic events (25% to 30% of strokes) and small vessel disease (25%)[35].

Large vessel disease seldom occurs in isolation, with neuroimaging evidence of small vessel disease being nearly ubiquitous in older adults. This may not always be clinically significant, however. Alzheimer's, Lewy body disease and other neurodegenerative pathologies may also coexist.

Small Vessel Disease

The term SVD refers to a group of pathological processes of different aetiologies affecting the small arteries, arterioles, venules and capillaries of the brain[3]. As these vessels cannot be visualized directly, the following parenchymal lesions resulting from pathology are commonly seen. SVD is much more common that large vessel disease, the most common cause of VCD and indeed, the most common neuropathology seen with ageing[3, 36, 37].

White Matter Hyperintensity

White matter hyperintensity (WMH) of presumed vascular origin[38], describes diffuse, confluent, white matter abnormalities, which are low density on CT and hyperintense on T2-weighted MRI and fluid-attenuated inversion recovery (FLAIR). Increasing sensitivity of MRI has resulted in less specificity and predictive validity of WMH, which can now be detected in more than 90% of older adults[39, 40] and nearly 50% of individuals in their late 40s.[41]

The major neuropathological features found in WMH in association with VCI include axonal loss, enlargement of perivascular spaces, gliosis, myelin loss and microglial activation[42]. These are caused by arteriosclerosis, lipohyalinosis, and fibrinoid necrosis of small vessels, in particular the long perforating arteries[43]. Recent data suggest that venous collagenosis is also associated with WMH[44]. WMH are most extensive in the periventricular regions and may extend to the deep white matter but spare areas protected from hypoperfusion, such as the subcortical U-fibers and external capsule, claustrum, and extreme capsule[45]. There is ongoing debate as to whether periventricular and deep white matter lesions are distinct in their etiology, risk factors and presentation[46] and there have been recent attempts to better examine the cognitive impact of regional WMH lesions by mapping onto strategic WM fiber tracts[47].

There is an association between WMH and cognitive and functional decline, particularly robust for those with progressive and more extensive, confluent lesions [48]. The cognitive domains of frontal-executive function and processing speed may be particularly affected [48, 49].

Diffusion tensor imaging (DTI) has revealed changes in tissue anisotropy and diffusivity in normal appearing white matter, thought to represent early microstructural changes before lesions are seen as WMH on structural imaging[50]. DTI may hold promise in the evaluation of VCD. There are a variety of DTI metrics, including the peak width of skeletonized mean (PSMD)[51], based on the variance in diffusivity of skeletonized white matter tracts may be more informative than conventional DTI measures.

Lacunes

Lacunes are defined by the STRIVE criteria as "a round or ovoid, subcortical, fluid-filled cavity of between 3 mm and about 15 mm in diameter, consistent with a previous acute small subcortical infarct or hemorrhage in the territory of one perforating arteriole"[38] . They are common in the general elderly population, with a prevalence of around 20% [52] and may be associated with VCD. There is no consensus on the specific number and location of the lacunes required for a VCD diagnosis, as lacunes are commonly asymptomatic [53].

Microinfarcts

Cerebral microinfarcts are a common pathological findings in the brains of the elderly both with or without dementia, including 62% in those with VaD in one report , and there is good evidence for their pathophysiologic importance in autopsy studies[54]. Estimated in size to range from 50 μm to 5mm, they were commonly thought to be invisible on gross neuropathological examination or MRI, being below visible image resolution[55]. Recent studies have shown them to be detectable on 3T scanning[56, 57] and although it is likely that only a small percentage of microinfarcts are being detected, there is some evidence that they are associated with cognitive impairment and dementia[57].

Dilated Perivascular Spaces

Infarctions should be distinguished from dilated perivascular spaces (PVS), also known as Virchow-Robin spaces, which are extensions of the fluid spaces that surround small blood vessels as they travel through the brain parenchyma. Although commonly microscopic and potential rather than real spaces, with high resolution MRI scanners, visible PVS are frequently seen on imaging and some studies have found near ubiquity, with prevalence up to 100%[58, 59]. There is ongoing debate as to the significance of visible PVS and the relevance of their size. Once thought to be a normal variant, there is increasing evidence for the association of dPVS with neurodegenerative and other cerebral pathology, in particular cognitive impairment[60, 61], ageing[59], cerebral SVD[59, 62] and Alzheimer's and vascular dementia[61, 63]. Hypothesized aetiologies include hypertension, obstruction, inflammation and atrophy[64].

Microbleeds

Cerebral microbleeds (CMB) are visualized as punctate hypointense lesions on paramagnetic-sensitive MRI sequences and correspond to small perivascular haemosiderin deposits, representing breakdown products from prior microscopic hemorrhages [65].

CMB are found in both cognitively normal and impaired individuals, and their prevalence increases with age from 7% at age 45-50 years to 36% at 80 years and older [66]. While their prevalence is highest in VaD (65-85%), CMB are also frequently found in AD (18-32%) and MCI (20-43%)[67]. The distribution of CMB in the brain differs according

to underlying pathology. CMB in the subcortical and deep regions are thought to be associated with arteriosclerotic/hypertensive small vessel disease and in contrast, CMB in lobar regions are more likely associated with cerebral amyloid angiopathy (CAA) [68, 69].

The relationship between CMB and cognition is inconsistent [70, 71] and more data are needed in relation to the standard measurement of CMBs, and their diagnostic and prognostic significance before CMBs are routinely applied in clinical assessments.

Hemorrhages

Cognitive deficits have been reported in 19% to 62% of patients following subarachnoid hemorrhage[72] and their severity is related to the severity of the hemorrhage. Multiple hemorrhages or hemorrhagic infarcts are often associated with VCD. CAA is a common cause[73] and other genetic disorders[74] and hypertension also play a role.

Hypoperfusion - Hippocampal Sclerosis

Hippocampal sclerosis (HS) is a pathological diagnosis, characterized by severe neuronal loss with reactive gliosis in the CA1 sector of the hippocampus. HS usually presents with slowly progressive memory impairment, resembling and often mistaken for AD. MRI often shows asymmetric hippocampal atrophy. The pathogenesis is multifactorial, with ischemic injury playing a major role[75].

Other Factors

Brain Atrophy

Both diffuse cortical and mediotemporal atrophy are associated with cognitive impairment[76]. Although commonly associated with neurodegenerative pathology, MTA may also result from vascular pathology[77] and reduced hippocampal volumes have been reported in VaD in the absence of AD pathology at autopsy[78].

Cerebral Amyloid Angiopathy

Cerebral amyloid angiopathy is a heterogeneous group of sporadic and, more rarely, hereditary diseases characterized by the progressive accumulation of amyloid proteins

in the vessel walls of leptomeningeal and cortical arteries, arterioles, capillaries and, less commonly, veins[79]. It is a frequent finding in the general elderly population, with approximately 50% of those over 80 years showing some amyloid deposition which may be asymptomatic[80]. Amyloid deposition can lead to weakening, potential rupture of vessel walls, or obstruction of the vessel lumen and subsequent ischaemia and present with a wide spectrum of clinical presentations. It can present with TIAs, stroke, seizures, migraine, and cognitive impairment and behavioral symptoms as a result of CMB , lobar hemorrhage, SAH and cortical infarction[80]. Its significance in a given patient with cognitive impairment is unclear and further examination of its relationship with clinical and imaging characteristics is needed[79].

Genetic Causes

VCD is uncommonly associated with a number of mendelian disorders[81] including cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Although relatively rare, CADASIL is the most common form of hereditary SVD and important as a pure model of VCD. This is due to an onset generally between the ages of 40 and 50 years, when comorbid AD pathology is rare. The use of cholinesterase inhibitors in those with CADASIL has shown statistically significant improvement in some measures of executive function, which provides a basis for cholinergic therapy for VCD[82]. Cholinergic mechanisms appear to play a critical role in cerebral perfusion[83]. The role of the apolipoprotein E (*APOE*) gene polymorphism in VCD is not clear although data have been presented, albeit inconsistently, for the association of the ε4 allele with WMH, VaD[84] and VCD[85].

The expression of dementia in an individual with CVD is also influenced by other factors including education and age[19, 37]. Higher education may represent greater brain reserve and be a non-specific protective factor for dementia[86]. Age may be seen as representing an accumulation of deficits over time and the body's response in terms of reparative processes. This frailty model has been applied to the presence of dementia, whether due to vascular pathology or other diseases[87].

Structural and functional connectivity

Cognitive function is widely distributed through the brain in a number of cortical/subcortical networks and the cognitive consequence of parenchymal injury from CVD may be mediated through perturbations to these networks[88, 89]. Structural networks can be examined through DTI-tractography and functional connectivity through fMRI BOLD techniques[90] using graph/network theory. Recent data suggest that SVD load is related to widespread disruption both to global integration and localized segregation with networks of lower density, connection strengths, and reduced network efficiency. Further, network efficiency mediates the expression of SVD independent of total SVD load[91, 92].

Interaction of vascular and neurodegenerative disease

CVD and AD pathology commonly coexist[93] and the presence of CVD worsens the expression of AD[94]. The interaction between the pathologies is complex and beyond the scope of this review. We point the reader to recent reviews[9, 95].

Figure 1.1: Schema of proposed VCD mechanisms. Multiple genetic, environmental and vascular risk factors lead to vessel pathology, CVD injury and subsequent network dysfunction and cognitive impairment through multiple pathways. Brain/cognitive reserve can moderate the expression of this. AD pathology interacts with CVD on a number of mechanistic levels.
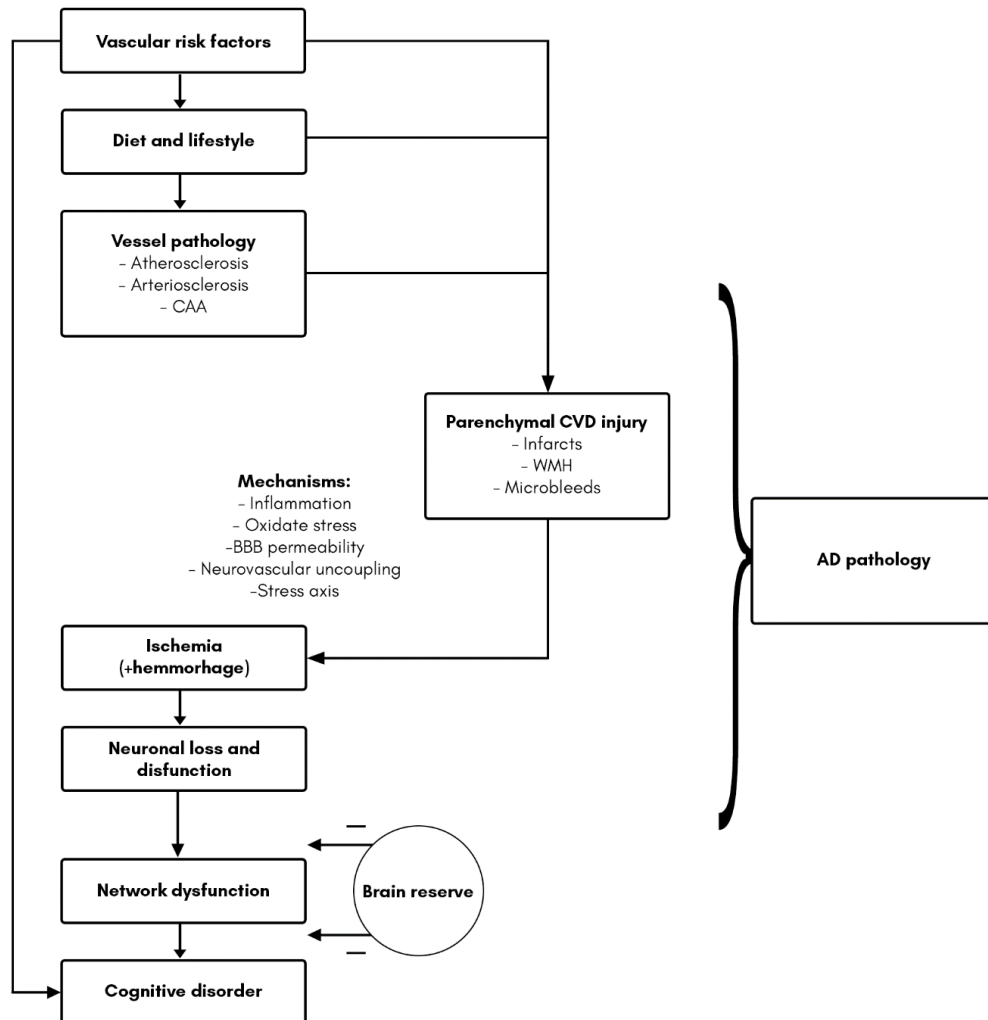
Subtypes of vascular cognitive disorders

Historically, VCD subtypes have been used inconsistently because overlap between the subcategories is common, reflecting the heterogeneity of pathology. The recent Vascular Impairment of Cognition Classification Consensus Study (VICCCS) guidelines[96], subdivided major VCD into four categories: PSD; subcortical ischaemic vascular dementia; multi-infarct (cortical dementia) and mixed dementias (with a variety of comorbid neuropathology). It is now well recognized that while SVD is common in cortical dementias, VCD may be exclusively due to subcortical vascular lesions[37] (where predominantly due to WMH, this has been referred to as Binswanger disease).

Diagnostic evaluation/clinical features

VCD is a clinical diagnosis. Making the diagnosis requires demonstrating that there is subjective concern about cognitive impairment, that there is objective evidence of cognitive impairment and establishing that the cognitive impairment is primarily due to a vascular cause[23] (see Table 1.2).

Subjective concern may originate from the patients themselves or knowledgeable informants (relative, caregiver, physician). A detailed account should be sought about the onset, progression and impact of cognitive impairment and the cognitive domains in which these difficulties occur. Compensatory mechanisms should be noted and whether the impairments cause significant impact in functioning.

A wide variety of cognitive deficits are seen in VCD. The classic description of MID was that of an acute stepwise or fluctuating decline in cognition, with intervening periods of stability and even some improvement[18, 97]. This pattern is temporally related to cerebral infarcts, hemorrhage, or vasculitis. The cognitive impairment is at its peak soon after a stroke and may show significant improvement over the next 3 months; persistence beyond this period is generally considered necessary for the cognitive disorder to be diagnosed[98]. A wide variety of other presentations are also possible[37]. The clinical presentation of subcortical ischemic vascular disease (SIVD) is a profile of a dysexecutive syndrome, accompanied by psychomotor slowing and attentional deficits with no (or minimal) memory impairment, commonly seen in the presence of subcortical lesions including white matter injury. This may be accompanied by

personality change and emotional lability. The Montreal Cognitive Assessment (MoCA)[99] is more likely to pick up relevant deficits than Mini-Mental State Examination (MMSE)[100] but full neuropsychological assessment is recommended and specific cognitive test batteries have been published[101]

Because of its heterogeneity, the progression of VCD shows considerable variability. Broadly, cognitive deterioration occurs at the same rate in VaD as AD[19]. Mean survival is reduced, due to cardiovascular comorbidities, with a mean survival of 3-5 years[102].

Risk factors

In addition to patient factors, such as increasing age and low education, there are a number of potentially modifiable risk factors for VaD[103]. These include mid-life hypertension, diabetes, hyperlipidemia, smoking, obesity, ischaemic heart disease and depression [104]. Stroke is a strong risk factor for VCD and pre-existing cognitive impairment is a risk factor for the expression of PSD[105].

Establishing a Predominantly Vascular Cause for the Cognitive Disorder

Assessment should include evaluation of vascular risk factors (e.g., hypertension, hyperlipidemia, diabetes mellitus, obesity, alcohol or tobacco use, physical inactivity), and evidence of CVD in the form of gait disturbance, urinary incontinence, and cardiovascular and cerebrovascular disease. The physical examination should therefore include assessment of the above, including examination of the cardiovascular system for evidence of arrhythmias or peripheral vascular disease and neurologic examination for focal neurologic signs, and assessment of gait initiation and speed [23, 101]. There are no specific recommended laboratory blood tests for VCD, but it would be reasonable to test for alternative reversible causes of cognitive impairment including B12, folate and thyroid function.

No biomarker is currently recommended in the assessment of VCD. The search for reliable biomarkers of VCD to aid diagnosis has been hampered by the heterogeneity of VCD and the overlap of neurodegenerative and vascular pathology[106]. One putative marker is the CSF albumin index as a marker of damage to the BBB[101]. Emerging

markers include carotid intimal-medial thickness and arterial stiffness, which are associated with arterial aging and may serve as risk markers of VCD[103].

Neuroimaging

Neuroimaging is critical for increased certainty in the diagnosis of VCD particularly with silent lesions and WMH. It is also necessary to rule out less common pathologies such as brain tumors or normal pressure hydrocephalus and to distinguish VaD from AD or frontotemporal degeneration as the cause of the cognitive impairment. MRI is preferred to CT as it is a more sensitive modality. The minimum radiological evidence of CVD pathology required to support the diagnosis is an ongoing question, particularly given the visualization of vascular pathology is technology dependent[2]. Given this, the recent STRIVE criteria have attempted to standardize the neuroimaging of SVD[38].

Traditional neuroimaging measures of CVD, such as WMH, are inconsistently correlated with cognition. There is therefore growing interest in using composite, multi-modal indices of CVD pathology (Paradise et al. Neurology, in press), which may provide more information than that obtained using a single pathology[107, 108]. Advanced and non-structural imaging modalities are also informative. Perfusion imaging may reveal abnormalities before they are seen as structural changes, amyloid imaging using positron emission tomography can differentiate AD from CVD, and DTI can assess microstructural changes[109, 110].

Neuropsychiatric symptoms

Given the injury to prefrontal brain regions, neuropsychiatric symptoms are common in those with VCD[111]. Depression, apathy and agitation are thought to be more common in VaD than AD[111-113]. Depression is the most extensively studied affective syndrome in VCD and post-stroke depression is common and, in clinical settings, figures of 21.6% for major and 21% for minor depression have been cited[114]. Psychotic symptoms are common in VaD, with one review[115] reporting that 37% of patients experienced psychotic symptoms, of which 19% to 50% experienced delusions, 14% to 60% visual hallucinations, and 19% to 30% delusional misidentification. Apathy is common in VCD, with the point prevalence in VaD calculated to be 33.8% and the prevalence in stroke patients ranging from 22.5% to 56.7%.[116]

Neuropathology

A definitive diagnosis of VCD requires neuropathological verification[43]. This will confirm the clinical or radiological evidence of vascular brain injury and detect lesions not easily identified with neuroimaging such as microinfarcts. It is also necessary to exclude significant non-vascular pathology which would rule out the diagnosis of VCD or at least render a diagnosis of mixed pathology more appropriate. There are a wide variety of pathologies seen, with signs of vessel wall modification (arteriolosclerosis, CAA) potentially leading to perivascular space changes and parenchymal damage, which manifest as either WM changes or microinfarcts[117].

Unfortunately, there is a lack of well validated and accepted neuropathological guidelines. This is due to the heterogeneity of CVD lesions, the unclear and overlapping definitions of CVD lesions and a lack of clinico-pathological correlation[118]. The recent VCING[119] criteria represent an attempt to standardize neuropathological assessment in VCD. Post-mortem neuropathology also cannot elicit the relationship between pathology and clinical presentation and despite recent data[117, 119, 120], it remains unclear which lesions are most associated with ante-mortem cognitive impairment.

Management

Prevention

With the failure of disease modifying drugs for dementia, there is increased emphasis on prevention of dementia[10]. Several large studies have recently reported that the incidence and prevalence of dementia is decreasing across Western countries[16, 17], perhaps reflecting better cognitive reserve and general health. The change in vascular risk factors across the population over time is nuanced, with better control of hypertension, hypercholesterolemia and smoking, but an increase in obesity and diabetes[104].

The evidence for modification of VCD risk factors has been summarized[103] with reasonable evidence that adequate physical activity, weight control, smoking cessation, and moderation of alcohol intake are advisable. Complex cognitive activity

has been examined as a protective factor against cognitive decline, although it may be nonspecific and unrelated to the cause of the cognitive decline[86]. Control of mid-life hypertension, hypercholesterolemia and hyperglycemia are recommended and because stroke poses a major risk for VCD, stroke prevention and its prompt treatment, followed by rehabilitation, including cognitive training, should be emphasized in any measures used to prevent VCD.

With regard to secondary prevention, there have been major advances in the treatment of acute stroke and the prevention of recurrent stroke, including treatment of risk factors, use of antiplatelet agents and anticoagulants, thrombolysis and endovascular thrombectomy. The use of neuroprotective agents in stroke patients has, however, been disappointing [121, 122].

Symptomatic Treatment

The effects of treatment in VaD are modest and no therapy has been approved by the FDA, Australian or European regulatory agencies. The use of cholinesterase inhibitors and the NMDA antagonist, Memantine have been examined, with only modest benefits seen in certain domains[19]. The gain was estimated to be about half that seen in AD[19], and thought to be of doubtful clinical significance[123].

Other drugs that have been tried in VaD include nimodipine, piracetam, huperzine A, cytidine diphosphocholine, and vinpocetine, but with no beneficial effects[103]. Cerebrolysin, a mixture of neurotrophic peptides derived from pig brains has shown early positive effects[124] but requires regularly intravenous infusions, limiting its use. Gingko biloba may also have beneficial effects[125]. Sertraline has been shown to have a beneficial effect on executive function in VCD in a small study[126].

Acknowledgements

1.4   Why does quantification of CVD matter?

Accurate quantification of CVD is required for several reasons. First, for an accurate diagnosis of dementia and subtype. For a diagnosis of VaD or VCI, a determination needs to be made that there is a vascular aetiology to the observed cognitive deficits. This is not always a straightforward task, given how common CVD is in the elderly population. The nature and severity of the cognitive deficits alone are rarely sufficient to confirm a vascular aetiology as there are no pathognomonic deficits in cognition. Although focal neurological deficits can be used as evidence of vascular pathology, more commonly, it is neuroimaging evidence of CVD burden that is utilised.

Table 1.4 outlines the neuroimaging requirements for the various vascular cognitive disorders, according to the mostly commonly used clinical and research diagnostic criteria. There is considerable heterogeneity in requirements, which leads to different diagnostic rates[21, 127]. In one cohort of ischaemic stroke patients[21] for example, the proportions fulfilling different diagnostic criteria for VaD ranged from 33% to 92%. Different neuroimaging requirements contribute to this variability. The VasCog criteria[23], harmonised with DSM-5[24] were an attempt to correct this.

Table 1.4: Neuroimaging requirements for common vascular cognitive disorders classification.

Diagnostic criteria	Diagnostic entity	Neuroimaging requirements
DSM-5	Vascular neurocognitive disorder	For 'probable' vascular neurocognitive disorder, clinical criteria supported by neuroimaging evidence of significant parenchymal injury attributed to cerebrovascular disease. Otherwise consider 'possible;' Evidence: one or more large vessel infarcts or haemorrhages, a strategically placed single infarct or haemorrhage (e.g., in angular gyrus, thalamus, basal forebrain), two or more lacunar infarcts outside the brain stem, or extensive and confluent white matter lesion
VasCog[23]	Major and minor VCD.	Presence of significant neuroimaging (MRI or CT) evidence of cerebrovascular disease (one of the following): (1) One large vessel infarct is sufficient for Mild VCD, and ≥ 2 large vessel infarcts are generally necessary for VaD (or Major VCD) (2) An extensive or strategically placed single infarct, typically in the thalamus or basal ganglia may be sufficient for VaD (or Major VCD)

		(3) Multiple lacunar infarcts (>2) outside the brainstem; 1-2 lacunes may be sufficient if strategically placed or in combination with extensive white matter lesions
		(4) Extensive and confluent white matter lesions (5) Strategically placed intracerebral hemorrhage, or Z2 intracerebral hemorrhages
ADDTC criteria for ischaemic vascular dementia[98]	Probable ischaemic vascular dementia	Two or more ischemic strokes or single stroke with temporal correlation with dementia And 1 infarct outside cerebellum in CT/ T1-weighted MRI. Extensive PV and deep WM lesions are *"thought to be associated with IVD"* [98]
ICD-11[128]	Dementia due to cerebrovascular disease	Nil specific specified: *"Evidence of the presence of cerebrovascular disease considered to be sufficient to account for the neurocognitive deficits from history, physical examination and neuroimaging"* [128].
NINDS-AIREN[129]	Probable Vascular Dementia	Multiple large-vessel infarcts or single strategically placed infarct. Multiple basal ganglia and white matter lacunes or extensive periventricular lesions [129].

ADDTC, Alzheimer's Disease Diagnostic and Treatment Centers; DSM, Diagnostic and statistical manual of mental disorders, ed 4; ICD-10, International Classification of Diseases, 11th version; NINDS-AIREN, National Institute of Neurological Disorders and Stroke Association Internationale Pour la Recherche et l'Enseignement en Neurosciences.

Second, accurate quantification of CVD burden may lead to improved prognostic information for patients with a diagnosis of VCD and improved estimation of risk for pre-symptomatic individuals. This could either be based on baseline CVD measurement, or longitudinal change in CVD. To return to WMH, results of cross-sectional association with cognitive impairment have been equivocal[7], but there is better evidence that progression of WMH is associated with cognitive decline[130] and dementia[131].

Third, a reliable estimate of CVD burden could be used as a biomarker, particularly in dementia trials. Dementia trials are long and expensive and an accurate surrogate marker for clinical phenotypes could help speed up trials and identify new potential therapies and disease pathways. Criteria for a reliable surrogate marker are i) the biomarker must be able to predict the future natural course of the disease; ii) the effect of treatment on the disease should be explained by the effect of treatment on the surrogate and iii), there should be evidence for a rate of progression that is fast enough to allow monitoring of treatment effects within a reasonable period of time[132, 133].

1.5   How do we currently assess cerebrovascular disease?

CVD can be assessed directly post-mortem, on ex-vivo tissue samples. It can also be visualised by a variety of neuroimaging techniques and inferred through measurement of fluid-based biological markers. Finally, cognitive measures have been used as a proxy for CVD.

The benefits and challenges of neuropathology are discussed in Chapter 1.7: Neuroimaging and neuropathology indices of cerebrovascular disease burden; A systematic review[2]. Whilst neuropathology allows detailed examination of a wide range of different vascular pathologies in better detail than any alternative method, the field is limited by lack of consensus criteria with unclear, overlapping definitions of CVD lesions and a lack of clinicopathologic correlation[119, 134, 135]. Without the current ability to sample the whole brain, the problem of selective sampling of certain regions remains and most obviously, short of brain biopsy, neuropathological examination has limited use in initial diagnosis and prognosis.

There are a range of potential CSF and blood-based biomarkers of vascular injury, reflecting a wide array of putative aetiological mechanisms. These include markers of blood-brain barrier (BBB) or endothelial dysfunction (CSF-serum albumin ratio, astrocytic protein S100β), markers of extracellular matrix breakdown, markers of subcortical neuronal degeneration and myelin damage (neurofilaments and CSF sulfatide), markers of inflammation and glial activation (CRP and various interleukins) and a range of other mechanisms. More established markers of Alzheimer's pathology (CSF Aβ42) also play a role in exclusion of alternative diagnoses. For recent reviews, please see [106, 136, 137]. There is no definitive marker for vascular injury and the field of biomarker development is challenged by the overlap of neurodegenerative and vascular pathology and the heterogeneity of pathologies seen.

The main limitation of using cognitive tests as a proxy for CVD is the lack of pathognomonic cognitive deficits seen with vascular cognitive disorder. Although SVD is often associated with deficits in attention and processing speed and executive function[1], this is not always the case, with deficits potentially seen in all cognitive domains, depending on the location and extent of lesions and other psychiatric and

medical comorbidities [23, 101]. Cognitive measures may also not be as sensitive to change as other biomarkers, important when considering their use as a proxy for underlying pathology in treatment trials. One study estimated a sample size (per arm) of 124 participants for change in WMH volume, compared to over 6000 participants when using executive function (at a 30% estimated effect size, per arm)[138].
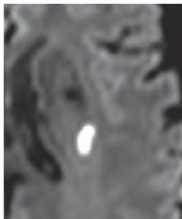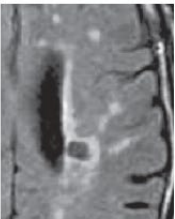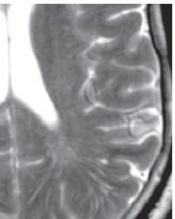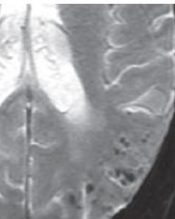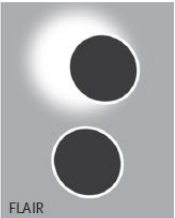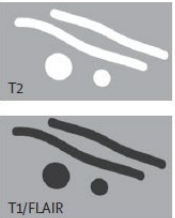
Currently then, neuroimaging may offer the most practical solution for biomarker use in routine clinical practice. Broadly, it offers several advantages over alternative methods. CT and increasingly MRI is commonly used in routine clinical practice and widely available. Most modalities are non-invasive and well tolerated by older individuals. Unlike neuropathology, neuroimaging allows examination of the entire brain. Finally, there are a wide range of structural and functional imaging modalities, allowing examination of a large number of vessel and parenchymal pathologies as well as underlying mechanisms[2, 38, 110, 139].

I will now proceed to examine current practice in the neuroimaging of CVD and examine the techniques and modalities assessed in more detail.

1.6 Current neuroimaging methods to quantify cerebrovascular disease

Numerous imaging techniques can be used to assess CVD and a non-exhaustive list includes structural CT and MRI, susceptibility-weighted MRI, diffusion weighted MRI, functional MRI which relies on global and regional blood oxygen level dependent imaging, CT and MRI angiography, perfusion imaging (including PET and SPECT approaches), imaging of the blood-brain barrier and connectome and network techniques. Many of these techniques are not routinely used clinically and please see [110, 140-143] for current reviews. The most common MRI markers of SVD are shown in Figure 1.2, along with their signal characteristics. Diffusion tensor imaging (DTI) techniques will also be discussed as well as approaches that combine information from multiple MRI markers, referred to as indices.

Figure 1.2: Common MRI markers of SVD. From Wardlaw et al.[38]

Abbreviations. DWI – diffusion weighted imaging. FLAIR – fluid-attenuated inversion recovery. SWI – susceptibility weighted imaging. GRE – gradient-recalled echo.

1.6.1 White matter hyperintensities.

WMH are white matter lesions that are visualised as bright, or hyperintense signals on T2-weighted MRI. The term is synonymous with leukoaraiosis[144] on CT; rarefication of the white matter, seen as darker than the surrounding area. Where deep grey matter is involved, the term subcortical hyperintensity is also used. WMH are the most common SVD pathology assessed on MRI. They are associated with CVD and vascular risk factors[38]. The underlying pathology is heterogeneous and ranges from slight disentanglement of the matrix to varying degrees of myelin and axonal loss[7], generally attributed to ischemic origin. The hyperintense signal visualised represents changes in white matter composition - altered water content in hydrophobic white matter fibres, due to pathology[145].

It is important to note that WMH are seen in a number of conditions and may represent several pathologies. This includes cerebral amyloid angiopathy (CAA) and less common in the elderly, multiple sclerosis and leukodystrophies. They are also commonly seen in Alzheimer's disease.

WMH are common in the elderly, and some degree of lesion is nearly ubiquitous in the elderly. In the Cardiovascular Health and Rotterdam studies, the prevalence of any WMH in the over 60s was 96% and 95 % respectively[39, 146]. WMH can be rated visually, with the commonly used Fazekas scale[147], which gives a score of 0-3 for severity of WMH in the periventricular and deep white matter regions. WMH can start as thin caps in the frontal or occipital horns of the ventricles, or small foci in the deep white matter, becoming more confluent, extensive and extending in the subcortical white matter as the lesions become more severe. WMH volume can also be calculated automatically, using a number of common imaging programmes, including techniques developed by our own group[40, 148]. Whilst global volume is the most common metric, segmentation can divide WMH into periventricular and deep volumes as well as into smaller regions of interest and lesions can be mapped on specific white matter tracts[47]. This region-based approach may be more informative, with WMH burden in strategic

white matter tracts explaining more of the variance in cognition than global WMH measures[149].

Many studies have demonstrated an association between cross-sectional WMH severity and impairment in either global or specific cognitive domain impairment[145], with a meta-analysis of 23 cross-sectional studies showing a robust, but small effect size of 0.10[150]. There is evidence for a stronger effect on longitudinal cognitive decline. A meta-analysis of 22 studies showed an increased risk of cognitive impairment and double the risk of dementia[11] and other studies have shown increased risk of AD more specifically[7, 12]. Although results have been mixed[13, 14], WMH severity has been associated with accelerated cognitive decline in established dementia[15]. WMH are most commonly associated with declines in attention and processing speed and executive function, though they may be associated with declines in any cognitive domain[7, 150].

The challenge for the clinician is interpreting these epidemiological results for an individual patient. Although group differences are found, there is considerable variability within individuals and clinically, it is common to see individuals with severe WM pathology who appear cognitively intact and conversely, cognitively impaired individuals with little WMH seen on scans.

Figure 1.3 provides an illustration of this, with two example participants from the Sydney Memory and Ageing Study[151] of a similar age and WMH global volume. The participant in panel A was aged 88, with a WMH volume of 67 cc^3 but with intact cognition, with a Global Cognition z-score of 1.29 and MMSE of 30. In contrast, the participant in panel B was aged 90, with a similar WMH volume of 69 cc^3, but had significant cognitive impairment, with a z-score in Global Cognition of -1.45 and MMSE of 23.

Figure 1.3: Illustration of variability of WMH with cognition.
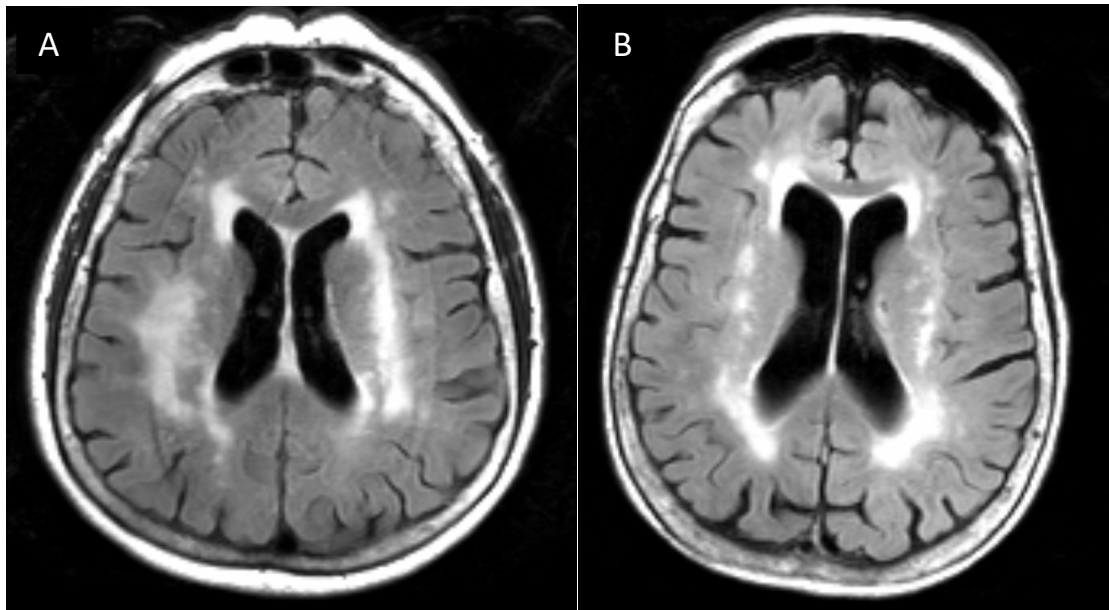
FLAIR axial scans of two example participants from the Sydney Memory and Ageing study[151].

It can therefore be difficult to use WMH severity alone as a marker of CVD burden and diagnostic support, in the absence of other supporting features for a typical vascular dementia (i.e., history of strokes, focal neurological deficits, typical cognitive deficits).

1.6.2 Other individual markers.

Compared to WMH, there have been fewer studies examining the association of both cerebral microbleeds (CMB) and dilated perivascular spaces (PVS) with cognitive impairment and dementia. These will be discussed in more detail below in Chapters 4, 5 and 6. The impact of lacunes and DTI metrics is discussed below.

1.6.2.1 Lacunes

Lacunes are small, subcortical, fluid-filled lesions that usually result from a small acute ischaemic infarct or haemorrhage in the territory of a perforating arteriole[38]. The overwhelming majority of studies have found a cross sectional association between lacunes and impairment in executive function, processing speed or global cognition [152-159]. Many of the studies did not find an association with memory however[155-157]. There have been fewer longitudinal studies, but these too find lacune number at baseline associated with decline in global and executive function[160]; lacune volume at baseline associated with decline in executive function (but not memory) [161] and incident lacunes over the study period associated with decline in executive function[162, 163], processing speed[130], or incident dementia[164].

A cross-sectional study, with 115 participants from a university dementia clinic did not find that lacune number of volume differentiated between diagnostic group cross-sectionally – normal controls, MCI or AD[165]. Another small cross-sectional study[166] of just 35 participants with lacunar stroke and leukoaraiosis did not find an association between the number of infarcts presents and cognition – either global or executive function or memory. Little description was given of the variability of lacune number in this cohort. And one longitudinal study [167] of 50 healthy older adults did not find an association with decline in memory or executive function, but this study only had 15 participants with lacunes, reducing its power.

1.6.2.2 Diffusion tensor imaging (DTI)

DTI is a sensitive MRI technique, based on the qualities of water diffusion. Alteration to tissue microstructure can be modelled in a tensor, a 3-dimensional ellipsoid representing net direction of diffusion. The most common patterns of diffusion change in damaged tissue are reduction in directionality (fractional anisotropy) and increase in the magnitude of directionality (mean diffusivity). DTI alterations can be detected even in normal appearing white matter, which are not picked up on conventional structural MRI. Several studies[51, 168-171] and a meta-analysis[172] have shown an association between DTI metrics and cognition across the spectrum of cognitive impairment. Further, that this association remains even after adjusting for WMH[168, 171] suggesting the DTI may be a useful technique for assessing early CVD. More recently, additional DTI metrics have been developed including peak width of skeletonized mean diffusivity (PSMD)[51], which was shown to be associated with processing speed in a diverse range of cohorts and outperformed traditional markers of SVD, including WMH and lacune volume[51].

1.6.3 CVD indices.

Given the frequently heterogenous nature of CVD, there have been recent attempts to combine information from several neuroimaging markers to form CVD indices[2]. The presumption is that using data from different markers and modalities will better capture the variability seen on neuroimaging and lead to more accurate associations with the phenotype being studied. This includes the recognition that normal appearing tissue on structural imaging may still be pathological, which can be revealed when interrogated by other modalities, e.g., DTI[173]. The attempt is to better model the "total CVD burden" and I will be examining the associations with cognition.

Chapter 1.7: Neuroimaging and neuropathology indices of cerebrovascular disease burden: A systematic review[2], reviews the current literature on CVD indices derived from both histopathological and neuroimaging indices. Importantly, it also outlines the challenges associated with development of a comprehensive CVD index:

First, there are a multitude of different lesion types seen in both the vessels themselves and the surrounding brain parenchyma. Many of these lesions are

common in the normal aged population, with few or variable clinical consequences, and so their significance and threshold levels for definition as a pathology remain uncertain. They may also represent several, potentially overlapping pathology, including different forms of SVD and neurodegenerative disease[5,6]. The visualisation of these lesions is technology dependent and may vary by MRI field strength and imaging parameters. Improved MRI resolution, for example, has rendered the detection of certain lesions, such as dilated perivascular spaces (dPVS), near ubiquitous.[7] Second, unlike large vessels, small vessels cannot currently be visualized directly in vivo. Neuroimaging of SVD therefore relies on the visualization of the consequences of the disease in the form of changes to the surrounding parenchyma. Third, there may be strong collinearity between certain CVD lesions based on similar underlying aetiology. The markers may also represent different timepoints on a pathologic continuum, i.e., the proposed evolution of small subcortical infarcts into white matter hyperintensities (WMH) or lacunes.[8] Fourth, there is variability in the outcome of any lesion. This may be based on the characteristics of the lesion itself, such as its size and location,[9] or due to other modifying/mediating variables such as cognitive reserve[10]. Fifth, CVD in the elderly is rarely found in isolation and the role of comorbid pathologies such as amyloid needs to be considered.[3]

1.7 Publication: Paradise MB, Shepherd CE, Wen W, Sachdev PS.

Neuroimaging and neuropathology indices of cerebrovascular disease

burden: A systematic review. Neurology 2018;91:310-320

Neuroimaging and neuropathology indices of cerebrovascular disease burden: a systematic review

Abstract

Objective:

We aimed to systematically review the literature on the use of both neuroimaging and neuropathological indices of cerebrovascular disease (CVD) burden, as estimation of this burden could have multiple benefits in the diagnosis and prognosis of cognitive impairment and dementia.

Methods:

MEDLINE and EMBASE databases were searched (inception to June 2017) to obtain and then systematically review all pertinent neuroimaging and neuropathology studies, where an index of CVD was developed or tested.

Results:

25 neuroimaging papers were obtained, which included just four unique indices. These utilized a limited range of CVD markers from mainly structural MRI; most commonly white matter hyperintensities (WMH), cerebral microbleeds and dilated perivascular spaces. Weighting of the constituent markers was often coarse. There were seven unique neuropathology indices, which were heterogeneous in their regions sampled and lesions examined.

Conclusions:

There is increasing interest in indices of total CVD burden which incorporate multiple lesions, as traditional individual markers of CVD such as WMH only provide limited information. Neuropathological indices are needed to validate neuroimaging findings. The studies clearly demonstrated proof of concept, that information from multiple imaging measures of CVD provide more information, including a stronger association with cognitive impairment and dementia, than that provided by a single measure. There has been limited exploration of the psychometric properties of published indices

and no comparison between indices. Further development of indices is recommended, including the use of data from diffusion tensor and perfusion imaging.

Introduction

Cerebrovascular disease (CVD) is a leading cause of morbidity and mortality. In addition to the well-known consequences of large vessel disease such as stroke and certain types of vascular dementia (VaD), the significance of small vessel disease (SVD) is being increasingly recognized. The clinical consequences of cerebral SVD are manifold, including lacunar stroke, vascular cognitive impairment or dementia, gait disturbance, urinary incontinence and neuropsychiatric symptoms[3]. The role of SVD in Alzheimer's disease (AD) is being increasingly recognized, with around 50% of cases having co-morbid small vessel pathology[4] and increasing evidence that vascular pathology has a deleterious effect on the expression and progression of dementia[174]. With the failure of disease modifying medications for AD, there is also increasing attention being placed on vascular pathology as a modifiable risk factor for dementia, with recent analysis suggesting that the rates of dementia could be dramatically reduced with modification of vascular risk factors including midlife hypertension, diabetes, obesity and smoking[175].

Despite the importance of CVD, there are no widely accepted criteria for the quantification of total cerebrovascular burden, either assessed via neuroimaging or with post-mortem neuropathological assessment. Producing an estimate of CVD burden presents a number of challenges. First, there are a multitude of different lesions types seen in both the vessels themselves and the surrounding brain parenchyma, from numerous etiologies[23, 176]. Many of these lesions are common in the normal aged population, with few or variable clinical consequences and so their significance and threshold levels for definition as a pathology remain uncertain. This is further complicated as the ability to visualize these lesions, particularly with neuroimaging, is technology dependent. Improved MRI resolution for example has rendered the detection of certain lesions, such as dilated peri-vascular spaces (dPVS) near ubiquitous[59]. Second, unlike large vessels, small vessels cannot currently be visualized directly in-vivo. Neuroimaging of SVD therefore relies on the visualization of the consequences of the disease in the form of changes to the surrounding parenchyma. Third, there may be strong collinearity between certain CVD lesions based on similar underlying etiology or the markers representing different time-points

on a pathological continuum, i.e., the proposed evolution of small subcortical infarcts into WMH or lacunes[38]. Fourth, there is variability in the outcome of any lesion. This may be based on the characteristics of the lesion itself, such as its size and location[177] or due to other modifying/mediating variables such as cognitive reserve[178]. Fifth, CVD in the elderly is rarely found in isolation and the role of co-morbid pathologies such as amyloid need to be considered[174].

There is increasing recognition from both the neuroimaging and neuropathology communities of the need for common definitions and criteria. The recent STRIVE criteria[38] reported definitions and common standards in the reporting of small vessel changes in neuroimaging. With neuropathology, several surveys of neuropathologists have highlighted differences in the categorization and recording of vascular pathology in the post-mortem assessment of dementia[11, 12]. Grinberg et al.[13] discussed the difficulties with establishing widely used neuropathological criteria for VaD, including it not being a single entity, unclear and overlapping definitions of CVD lesions and a lack of clinicopathological correlation. The recent vascular cognitive impairment neuropathology guidelines (VCING)[119] were developed using the Delphi consensus method and subsequent analysis.

An accurate neuroimaging estimate of total CVD burden could have several important applications. First, it could help clinicians better diagnose and subtype cognitive impairment and dementia as well as provide important prognostic information. Second, it could be used for stratification of individuals in clinical and genetic studies. Third, an accurate imaging index of CVD burden could be used as a surrogate marker for clinical trials. Currently, treatment trials in CVD are expensive and time consuming as clinical outcomes such as cognitive impairment and dementia may take many years to develop. There is therefore an interest in the use of markers which would allow researchers to extrapolate long term outcomes such as disease progression or treatment efficacy on the basis of short-term changes[133].

A neuropathological index of total CVD burden is necessary to provide the "gold standard" validation to imaging and clinical studies and may be more informative than the use of a single neuropathological lesion. A clinician must take all markers of disease to better understand the overall burden of CVD. In the last few years, there

have been attempts to develop both neuroimaging and neuropathological indices which use several features of CVD to give a better estimate of total CVD burden than could be provided by one or two features alone.

Aims/objectives

The aim of this systematic review was to examine the relevant published literature with regard to neuroimaging and neuropathological indices of CVD; to summarize the current state of the literature and suggest potentially useful ideas for further research.

Methods

Study identification:

Two separate systematic reviews of the neuroimaging and neuropathology literature were performed using the databases MEDLINE and EMBASE (from inception until June 2017). For the neuroimaging systematic review, we searched using the following key words which were also mapped to the databases' relevant search term vocabulary (i.e., MeSH headings in MEDLINE, Medtree term in EMBASE): cerebrovascular disease; cerebral small vessel disease; MRI; white matter hyperintensities; leukoaraiosis; lacune; subcortical infarct; microbleed; perivascular space; model; index; scale; rating and score. For the neuropathology systematic review, we searched using the following key words which were also mapped to the databases' relevant search term vocabulary: histopathology; neuropathology; histology; pathology; cerebrovascular disease; small vessel disease; multi-infarct dementia; vascular dementia; vascular cognitive impairment; model; index; scale; rating and score (figures 1.4 and 1.5). Reference lists of all relevant articles were also searched for additional studies.

Figure 1.4: PRISMA[179] flow diagram - neuroimaging


```
Identification

  Number of records identified          Number of additional records
  through a systematic search           identified through other sources
          (N=3098)                                  (N=0)

                   Number of records after duplicates removed
                                    (n=1914)

Screening

  Number of records                    Number of records excluded after
  screened (n=1914)                    reading title (n=1426)
                                                     ↓
                                       Number of records excluded after
                                       reading abstracts (n= 488)

Eligibility

  Number of full-text                  Number of articles excluded on
  articles assessed for                reading full-text (n=27):
  eligibility (n=52)
                                       • Conference abstract (n=6)
                                       • Not meeting inclusion/exclusion
                                         criteria (n=21)

  Number of articles                   Number of articles
  assessed for quality                 excluded on critical
          (n=25)                       appraisal (n=0)

Included

                   Number of articles included

                                  (n=25)
```

Figure 1.5: PRISMA[179] flow diagram - neuropathology

Stage	
Identification	Number of records identified through a systematic search (N=1878) — Number of additional records identified through other sources (N=2)
	Number of records after duplicates removed (n=1495)
Screening	Number of records screened (n=1495) → Number of records excluded after reading title (n=1352) ↓ Number of records excluded after reading abstracts (n= 114)
Eligibility	Number of full-text articles assessed for eligibility (n=30) → Number of articles excluded on reading full-text (n=19): • Not meeting inclusion/exclusion criteria (n=19)
	Number of articles assessed for quality (n=11) → Number of articles excluded on critical appraisal (n=0)
Included	Number of articles included **(n=11)**

Selection criteria:

For the neuroimaging and neuropathological systematic reviews, inclusion criteria were:

- Two or more CVD neuroimaging or neuropathological markers were required to be concurrently assessed to construct an index of some or all aspects of CVD
- Human studies with no age or sex requirements.
- Studies were required to have a clinical or neuropathological outcome to which the imaging index was related or a clinical or neuroimaging outcome to which the neuropathology index was related.
- There was no restriction on the diagnosis, cognition or CVD burden of the cohort.

Exclusion criteria were:

- If the index produced a binary indication of the presence or absence of CVD, rather than an index of severity.
- If the method was not sufficiently detailed to ascertain how the index was constructed/operationalized.
- Conference abstracts not followed up by full length publications.
- Studies not published in English.

Results

Neuroimaging:

There have been just four unique indices from the 25 papers where a multi-modal MRI index of CVD was developed or utilized. Table 1.6 reports a summary of the four indices, two of which were developed over several papers. Two of these indices assessed SVD severity[180] (including cerebral amyloid angiopathy (CAA) [181]) and two examined CVD as a whole, incorporating measures of both large and SVD[108, 182]. All indices contained between four and six neuroimaging markers, in the following order of descending frequency of use: WMH (in 4 indices), cerebral microbleeds (CMB) (3), lacunes (3), dPVS (2), acute cortical infarct (2), large vessel stenosis (2), global cortical atrophy (1), superficial siderosis (1).

Table 1.6. Summary table: MRI indices of CVD

Study lead author	Characteristics	MRI markers of CVD	Outcome measure(s)
Klarenbeek et al.[180]	122 ppts with first-ever lacunar stroke	5-point SVD ordinal scale (0-4) based on: lacune; extensive WMH; deep CMB; dPVS in the basal ganglia	Cross-sectional assoc. with blood pressure
Huijts et al.[183]	189 hypertensive or lacunar stroke ppts		Cross-sectional assoc. with global cognition and specific cognitive domains
Staals et al.[107]	461 ppts with lacunar or non-disabling cortical stroke		Cross-sectional assoc. with vascular risk factors, stroke subtype and cerebral atrophy
Staals et al.[184]	678 ppts from healthy ageing cohort		Cross-sectional assoc. with global cognition, processing speed and memory
Kandiah et al.[182]	209 ppts with mild stroke	15-point risk score based on: age, education, acute cortical infarcts, WMH, lacunes, global cortical atrophy, and intracranial large vessel stenosis	Post-stroke cognitive impairment at 6 month follow up

Xu et al.[108]	305 cases and 94 controls from memory clinic	Weighted 4-category cerebrovascular disease burden score: None/very mild (0); Mild (1); Moderate (2) and Severe (3). Based on: cortical stroke; intracranial stenosis; multiple lacunes; multiple CMB; moderate to severe WMH	Cross-sectional assoc. with global cognition and specific cognitive domains
Xu et al. [185]	863 ppts from population-based study		Cross-sectional assoc. with dementia, global cognition and specific cognitive domains
Charidimou et al.[181]	105 ppts with CAA	7-point SVD ordinal scale (0-6) based on: Lobar CMB; cortical superficial siderosis; centrum semiovale dPVS; WMH.	Retrospective cross-sectional assoc. with CAA-associated vasculopathic changes and symptomatic intracerebral hemorrhage

Abbreviations: ppts – participants; CAA - cerebral amyloid angiopathy; CMB – cerebral microbleeds; dPVS – dilated perivascular spaces; WMH – white matter hyperintensities.

With regard to the weightings of the constituent variables, the Staals et al.[107, 180, 183, 184] index used four markers from the literature, with one point gained for the presence of lacunes (≥1), microbleeds (initially only deep CMB[13, 17] and then modified to include any CMB[107, 184]) (≥1), moderate to severe dPVS in basal ganglia as operationalized by a semi-quantitative scale[62] and extensive WMH (periventricular severity 3 or deep WMH severity 2-3[147]). dPVS in basal ganglia and deep CMB were selected as these are most associated with SVD and WMH level was chosen as these WMH Fazekas scores were related to SVD in a previous clinicopathological study[186]. Huijts et al.[180] acknowledged that though generated through expert discussion, these cut-points were somewhat arbitrary. Charidimou et al.[181] generated an index based on pre-specified cut-offs and weighting, based on existing literature. Their index of four markers was weighted by the severity of lobar CMBs – one point for 2-4 CMB and two points >5 as well as severity of cortical superficial siderosis (disseminated two point and focal one point). A further point was gained for WMH and moderate to severe centrum semiovale dPVS. Kandiah et al.[182] developed their risk score using regression β-coefficients from multivariate analysis. This model was then tested on a validation cohort. Xu et al.[108] reported that global and domain-based neurocognitive scores were disproportionally influenced by severity of WMH and this effect may be more prominent than for lacunes. β-regression coefficients were used to operationalize their index of CVD burden, with WMH afforded a greater weight than other CVD indicators.

Several studies investigated the correlations between neuroimaging CVD markers. Xu et al.[185] reported that the presence of WMH was significantly correlated with lacunes and CMB, that multiple lacunes were associated with CMB and cortical infarcts and that intracranial stenosis was associated solely with cortical infarcts. In a study of those at risk for SVD, Huijts et al.[180] found where only a single neuroimaging marker occurred, it was most likely to be a lacune (44%), followed by a WMH (32%). If two markers co-occurred the most likely combination was a lacune and dPVS (49%), followed by a lacune and WMH (18%). In a study of lacunar stroke patients, Klarenbeek et al.[180] found where only a single neuroimaging marker occurred, it was most and equally likely to be a lacune or WMH (24%). If two markers co-occurred the most likely combination was a lacune and dPVS (54%), followed by a WMH and dPVS (22%).

None of the four indices reported inter-rater or intra-rater reliability of the index as a whole although the Staals et al.[107] index reported substantial to excellent agreement between raters on individual MRI markers of SVD: WMH and dPVS[180]. Where a dichotomous outcome was assessed, further psychometric properties could be assessed. Assessing post-stroke cognitive impairment at six months[182], a 15-point risk score had a ROC area-under-the-curve of 0.83 (95% CI: 0.77–0.88). Using a cut-off of ≥7 was 73.21% accurate, and had a sensitivity of 82.05%, specificity of 67.94%, positive predictive value (PPV) of 60.38%, and negative predictive value (NPV) of 86.41%. This was reliable in a second validation cohort[182]. Using a cut-off of moderate-severe CVD from their four level categorical risk score produced a sensitivity of 92.1% for a diagnosis of dementia in a community dwelling cohort[185]. In one study of SVD[184], latent variable modelling analysis was used to confirm that the constituent MRI features in Staals et al. [184] model were indicative of an underlying latent variable – overall SVD state.

Table 1.7 reports summary information of the subsequent 17 studies that have utilized these indices in a diverse group of cohorts. The majority of studies included participants with an expected high degree of CVD burden; nine of ischemic stroke or transient ischemic attack (TIA) cohorts; two cohorts with hypertension, one CAA cohort and one with vascular mild cognitive impairment (MCI). There was considerable heterogeneity in the clinical/pathological outcomes assessed and in methodology with eight studies assessing longitudinal associations. The operationalization of the Staals et al.[107] index varied across studies, with four rating scales[59, 62, 187, 188] used to determine dPVS severity.

Table 1.7. Summary of studies utilizing an MRI index of CVD.

Study lead author	Characteristics	Neuroimaging index utilized	Outcome measure(s)
Cleutjens et al.[189]	55 ppts with COPD	Staals et al.[107]	Cross-sectional assoc. with high functioning vs low functioning COPD patients
Xiao et al.[190]	413 first-ever acute lacunar stroke ppts	Staals et al.[107]	Cross-sectional assoc. with chronic kidney disease severity
Field et al.[191]	700 ppts from healthy ageing cohort[a]	Staals et al.[107]	Retrospective longitudinal assoc. with early life factors inc. IQ, deprivation and education.
Song et al.[192]	737 patients with ischemic stroke	Staals et al.[107]	Cross-sectional assoc. with presence vs absence of aortic atheroma
Uiterwijk et al.[193]	130 hypertensive patients	Staals et al.[107]	Longitudinal 4 yr assoc. with cognitive decline – global cognition and specific cognitive domains
Vilar-bergua et al.[194]	975 ppts with hypertension,	Staals et al.[107]	Cross-sectional assoc. with renal function
Wiseman et al.[195]	51 patients with SLE, 51 controls, 51 stroke pts.	Staals et al.[107]	Cross-sectional assoc. with diagnostic group.

Arba et al.[196]	115 thrombolysed ischemic stroke ppts	Modified Staals et al.[107]	Longitudinal, 90 day association with disability and functional dependency
Boulouis et al.[197]	261 ppts with probable CAA	Charidimou et al.[181]	Cross-sectional assoc. with diagnostic group – stroke like symptoms vs. cognitive complaints
Chen et al.[198]	687 ppts with ischemic stroke	Staals et al.[107]	Longitudinal, 72 hour association with early neurological deterioration
Kim et al.[199]	72 ppts subcortical vascular mild cognitive impairment	Staals et al.[107]	Longitudinal, 3 years assoc. with differences in APOE genotype diagnostic groups.
Molad et al.[200]	266 first-ever stroke or TIA ppts (n = 266	Staals et al.[107]	Longitudinal, 1 year assoc. with global cognition and specific cognitive domains
Pinter et al.[201]	678 ppts from healthy ageing cohort[a]	Staals et al.[107]	Cross-sectional assoc. with gait speed, chair-stands and balance
Song et al.[202]	1096 ppts with acute ischemic stroke[b]	Staals et al.[107]	Retrospective longitudinal association with date and cause of death. Mortality rate.
Song et al.[203]	170 ppts with suspected obstructive sleep apnea	Staals et al.[107]	Cross-sectional assoc. with severity of sleep apnea
Yang et al. [204]	210 patients with first-ever acute lacunar stroke	Staals et al.[107]	Cross-sectional assoc. with early renal impairment (serum Cystatin C level)

| Zhang et al. [205] | 374 patients with first-ever lacunar stroke | Staals et al.[107] | Longitudinal, 3 month association with post-stroke depression, presence vs. absence |

[a] Same cohort as Staals et al.[184] [b] Same cohort as Song et al.[192]

Abbreviations: ppts – participants; COPD – chronic obstructive pulmonary disease; CAA - cerebral amyloid angiopathy; TIA – transient ischemic attack.

The CVD indices were associated with global cognition, but not consistently with memory[183, 184, 193], executive function[183] or processing speed[184]. The association of the CVD indices with heterogeneous clinical phenotypes was more variable but higher burden on an index was *not* associated with COPD[189], specific early life factors[191], early neurological deterioration[198], or specific causes of mortality following an ischemic stroke[202]. No index was designed to measure progression of CVD burden and no study repeated a CVD index measurement at two or more timepoints to determine if the index was sensitive to change in CVD burden.

Multiple studies demonstrated that a composite index, but not an individual marker, was associated with cognition[185] and that greater scores (accumulation of MRI markers of CVD) were associated with greater cognitive decline[108, 182-184].

Histopathology:

Table 1.8 reports the summary of the 11 papers where a neuropathology index of CVD was developed or tested. These represent seven unique indices developed to rate the neuropathological burden of CVD, or an aspect thereof, from two or more constituent neuropathologies. These indices assessed CV parenchymal pathology (in 2 studies), CV pathology generally (2), SVD (3), a macrovascular lesion score (2) and a vascular score based on presence of cortical microinfarcts and basal ganglia and thalamic lacunes (2). The size of the studies ranged from 61 to 190 decedents and variously included cases with antemortem diagnoses of VaD, subcortical ischemic vascular disease, dementia with Lewy bodies, AD, mixed dementia and cognitive normal/ MCI cases and elderly individuals without significant AD or macrovascular pathology.

Table 1.8. Summary table: Neuropathology indices of CVD

Study lead author	Characteristics	Neuropathological markers of CVD	Outcome measure(s)
Esiri et al.[36]	18 NC, 19 CVD without dementia, 24 VaD	Semi-quantitatively 0-3 ordinal scale of SVD based on: widening of PVS, hyaline thickening of arteriolar walls, myelin pallor, nerve fiber attenuation/loss with gliosis.	Dementia diagnosis
Gold et al.[206]	72 cases without significant AD or macrovascular lesions.	0-6 vascular score based on: cortical microinfarcts, and basal ganglia and thalamic lacunes	Cognitive status (CDR)
Chui et al.[207]	79 cases with subcortical ischemic vascular disease and AD	0-300 cerebrovascular parenchymal pathology scores based on: number and location of cystic, lacunar and microinfarcts in grey and white regions and white matter demyelination.	Cognitive status (CDR) and ApoE genotype
Gold et al.[208]	156 cases with AD pathology	0-30 vascular score based on: cortical microinfarcts, and basal ganglia and thalamic lacunes	Dementia diagnosis and subtype
Strozyk et al.[120]	190 cases with diagnoses of dementia, AD and VaD	Macroscopic vascular lesion score (0-6) based on: large infarcts, lacunar infarcts (0, 1, and ≥2), and leukoencephalopathy (none, mild, and moderate-to-severe)	Dementia diagnosis and subtype

Smallwood et al.[209]	70 cases with CVD, excluding significant AD pathology	0-12 SVD pathology score based on: pallor of myelin staining, myelin loss, loosening of parenchymal tissue extending in places to cavitation, and dilation of perivascular spaces	Cognition (MMSE and CAMDEX)
Deramecourt et al.[117]	135 dementia cases of mixed pathology	0-20 vascular score based on: vessel wall modification (arteriolosclerosis and amyloid angiopathy), perivascular haemosiderin leakage, perivascular space dilatation, myelin loss and cortical micro- or large infarcts.	Neuropathological diagnoses – VaD; AD; DLB
Jung et al.[210]	16 subcortical ischemic vascular disease, 20 AD, 10 mixed pathology and 17 NC.	Chui et al.[207] index	Neuropathological diagnosis – Vascular disease; AD; mixed pathology; NC
Esiri et al.[211]	161 AD cases	Smallwood et al.[209] index	Cognitive scores (MMSE)
Skrobot et al.[119]	113 cases without significant neurodegenerative disease	3 element model based on: presence of moderate/severe occipital leptomeningeal CAA, moderate/severe arteriolosclerosis in occipital white matter, and ≥ 1 large infarct	Cognitive impairment (dementia or MCI)
Ezzati et al.[212]	62 cases without dementia	Strozyk et al.[120]	Cognitive decline - Blessed Information

	Memory concentration score

Abbreviations: NC – normal controls; CVD – cerebrovascular disease; AD – Alzheimer's dementia; VaD – Vascular Dementia; DLB – Dementia with Lewy Bodies; CAA - cerebral amyloid angiopathy; SVD – small vessel disease; PVS –perivascular spaces; MCI – mild cognitive impairment; CDR - Clinical Dementia Rating scale; MMSE – mini-mental state examination; CAMDEX - Cambridge Mental Disorders of the Elderly Examination; ApoE - Apolipoprotein E; CVDPS - cerebrovascular parenchymal pathology scores.

The choice of markers/pathology was less homogenous than for the neuroimaging studies above. Studies used between two items and seven constituent pathologies in their index. The following markers are ordered in descending frequency of use: white matter demyelination/pallor (in 9 studies), lacunar infarct (5), perivascular space dilatation (4), cortical microinfarcts (4), large (including cystic) infarct (4), arteriolosclerosis (3), amyloid angiopathy (2), perivascular haemosiderin leakage (1), hippocampal sclerosis (1), cortical infarct (1).

All studies examined the association of neuropathological CVD burden with dementia diagnosis, dementia subtypes or cognition. There was a heterogeneity of standardized regions sampled, ranging from just four regions[117] - frontal, temporal, hippocampus and basal ganglia (largely chosen because they represent relevant systems involved in cognition and receive blood from each major cerebral artery) , to over 15, with the neocortex, hippocampus, basal forebrain, basal ganglia, thalamus, mid-brain, pons, medulla and cerebellum sampled[120]. Where specified, most studies also examined just one hemisphere, as CVD distribution was thought to be reasonably symmetrical[36].

Few studies assessed collinearity or interactions between neuropathologies. Deramecourt et al.[117] assessed the distribution and frequency of individual CV lesions and reported vessel wall modifications as the most common pathology. They postulated a staging system for CVD and suggested that initial vessel wall modification (either arteriolosclerosis, CAA or both) could lead to perivascular space changes and parenchymal damage, which manifest as either WM changes or microinfarcts. The weighting of the constituent neuropathologies were then based on the frequency and presumed order of these lesions. Several SVD studies[36, 209, 211] used a semi-quantitative scale to reflect the presumed progression of microvascular disease, often with visual aids. The majority of studies included pathological markers based on a-priori knowledge without an explicit description of weighting of individual pathologies. Two studies examined the univariate associations of lesions with cognitive impairment to help construct an index. Skrobot et al[119] found that seven of their 14 agreed upon neuropathologies were associated with cognitive impairment. These were then entered into a multivariable regression model to identify which combination of pathologies was most strongly associated with cognitive impairment and they reported

that a single pathology predicted cognitive impairment with 60-65% accuracy, but using the best three predictors increased this to 78%. Further, that having either one, two or three of these pathologies predicted cognitive impairment with 38%, 75% and 95% accuracy respectively. Strozyk et al.[120] reported that all their macrovascular lesions were associated with VaD: large infarcts, lacunar infarcts and leukoencephalopathy. However, when considered together, only the presence of large infarcts remained significant.

Discussion

Neuroimaging studies have demonstrated the feasibility of using composite indices of total cerebral burden in predicting cognitive decline and dementia in a range of cohorts. They support the hypothesis that a composite index provides more information than a single variable has face-validity; that although there is significant overlap and collinearity between different neuroimaging features, the use of multiple markers will capture information not found in a single variable. There is less evidence to suggest that multiple neuropathological lesions are more strongly associated with cognitive decline or dementia than single lesions[119, 120].

When constructing a potential index, a balance has to be found between ease of use and accuracy. The most replicated Staals et al.[107] four item scale was intended for clinical use and can be calculated quickly and easily from routinely collected information. However, as the authors point out, its use of dichotomized cut-points for constituent variables could lose information and power. The psychometric properties of the indices have been insufficiently explored. In particular, the inter- and intra-rater reliability of a complete index have not been assessed and nor has there been an exploration of the relative merits of different indices, or a comparison with the psychometrics of a single pathology, such as WMH. There is insufficient data to make any recommendations as to the optimal composition of an index – either in the number or type of measures.

Neuropathological indices of CVD serve as a vital validation to neuroimaging and clinical studies. They can also provide additional information to that obtained on neuroimaging including a) CAA pathology, which is harder to visualize on

neuroimaging, relying on indirect signs such as superficial siderosis or microbleed distribution and b) cortical microinfarcts, the importance of which are being increasingly recognized[213] but which cannot be easily assessed using MRI. Neuroimaging in turn, has several advantages when assessing the burden of CVD. It is easier to assess whole brain pathology, which would be impractical for post-mortem assessment, which generally focus on specific regions. Equally, it can assess pathology through non-structural techniques, such as diffusion tensor imaging (DTI), which may not be replicable by histopathology.

None of the neuroimaging indices included DTI or perfusion imaging. DTI allows quantification of microstructural tissue alternations, which may be invisible on conventional structural MRI markers and therefore may provide additional information about the total CVD burden. There is ongoing debate as to whether DTI is superior to conventional SVD markers[51]. There are multiple technologies to estimate cerebral blood flow (CBF) using both endogenous or exogenous tracers. Methods such as arterial spin labelling allow relatively rapid quantification of CBF without a radioactive tracer using standard MRI technology. There is a strong association between CBF and SVD[214]. The question remains whether reduced perfusion is a cause or consequence of SVD[215] but longitudinal studies are starting to address this question and recent studies have started to examine the association of CBF with clinical phenotypes such as dementia diagnosis[216]. This study reported the association between hypoperfusion and dementia was independent of other markers of SVD and perfusion imaging may prove to be an important component of future CVD burden indices.

There have been three approaches to deal with the issue of collinearity between markers and their subsequent weighting. First, the inclusion of markers and cut-points based on a-priori research information from previous studies and expert consensus opinion. Second, a modification of this based on an iterative approach/sensitivity analysis. Third, the inclusion of markers and weighting of factors a-posteriori, based on the regression coefficients from multivariate analysis from within the study. Different approaches have advantages and disadvantages. The use of a-priori information links the study with existing knowledge and supports putative biological mechanisms. The

use of more theoretically neutral approaches allows greater freedom in the inclusion of novel features or measures of CVD. To avoid potentially spurious results however, if this second approach is used, a validation cohort should be utilized.

Only one study[181] of a CAA cohort examined the association of neuroimaging findings with histopathologically defined CVD, the gold standard of validation. There has been insufficient validation of neuroimaging findings with neuropathology, which can involve technical challenges. First, access to post-mortem brain tissue may not be readily available. Second, there is generally a time lag between an in-vivo MRI scan and a post-mortem examination in which unknown changes in CVD may have occurred. This can be addressed to some extent by post-mortem MRI, but very few brain banks have the resources to make this a routine process. Third, unlike AD and despite an increasing recognition for the need for consensus on vascular burden in brain autopsy studies, a common standard has not yet emerged and there is no consensus as to the best way to rate cerebrovascular neuropathology. Currently, there are no widely accepted criteria for neuropathological diagnosis of VaD or VCI[119] and surveys of neuropathological assessment have found widespread variation in definitions, sampling procedures and interpretation of vascular pathology. To assist with utilization, several neuropathology scales included the use of guide images[117, 209, 211] or a scoring template[207].

One of the primary motivations for the development of a total cerebrovascular burden index is in its potential use as a surrogate marker for clinical trials. Fazekas et al.[132] and Schmidt et al.[133] proposed criteria for such trials suggesting that it must be able to predict the future natural course of the disease, that the effect of treatment on the disease should be explained by the effect of treatment on the surrogate and that there should be evidence for a rate of progression that is fast enough to allow monitoring of treatment effects within a reasonable period of time. There is increasing evidence that a composite index of CVD burden would fulfil these criteria and may offer advantages over single, conventional MRI markers.

The main limitation of this systematic review was that we only included studies published in English. The studies were also sufficiently heterogeneous that a meta-analysis could not be conducted.

In conclusion, advances in MRI technology and the availability of large neuroimaging cohorts have allowed the development of neuroimaging composite indices of cerebrovascular pathology, which may offer several advantages compared to single or conventional MRI CVD features. They may more accurately represent the underlying CVD pathology and be more sensitive to change. Future studies should continue to develop and test novel indices as well as replicating existing indices in different populations and in longitudinal cohorts. In particular, indices should examine the weighting of constituent variables with increased sophistication and examine the significance of CV lesion location. The relationship between lesion location and cognition is complex and may only be significant for certain lesions such as WMH and lacunes[149]. The contribution of other putative markers of CVD derived from non-structural sequences, such as perfusion and spectroscopic imaging also require consideration. Currently, there is insufficient data to recommend one CVD index over another and further clinico-patho-imaging correlation studies are required to advance this important field.

1.7.1 Update to Neuroimaging and neuropathology indices of cerebrovascular disease burden: A systematic review. Neurology. 2018. 91(7): 310-320.

Method:

The 2018 systematic review was updated to capture any new neuroimaging indices developed since publication. The updated review did not cover neuropathological indices. The same search methodology was used as for the original publication with the same selection criteria. MEDLINE was searched for key words, which were also mapped to the databases' relevant search term vocabulary (i.e., MeSH headings), resulting in the following search syntax:

(MRI.mp. or nuclear magnetic resonance imaging/) and (cerebrovascular disorder.mp. or cerebrovascular disease/ or small vessel disease.mp. or leukoaraiosis/ or leukoaraisosis.mp. or white matter hyperintens*.mp. or lacunar stroke.mp. or lacunar stroke/ or lacun*.mp. or microbleed.mp. or subcortical infarct.mp. or perivascular spac*.mp. or perivascular space/) and (model or index or scale or rating or score).ab.

These results were then limited to papers published in English between 1st June 2017 and 17th November 2020, which were not conference abstracts. This produced 944 results. After reviewing the titles, 426 papers remained. After reading abstracts, 78 papers remained. Upon reading the manuscripts, two papers[199, 201] had been included in the original systematic review and were excluded and 13 manuscripts were rejected as they did not fulfil selection criteria (including two duplicate studies). Two additional papers, not picked up by the systematic review criteria were then added, leaving a final 65 papers where a multi-modal MRI index of CVD was developed or utilized in the update.

Results:

Despite the large number of studies since the original review was published, the majority of studies explored the associations of a narrow range of existing scales, or used minor modifications to these scales. Table 1.9 reports a summary of the eight new indices included in the review. Five studies[217-221] used some combination of the four common SVD features – WMH, lacunes, PVS and microbleeds. Dickie et al.[222] developed an overall Brain Health Index, with a novel automated approach, using

several MRI sequences to classify voxels into healthy or pathological tissue. This index is not specific to CVD, but did correlate with an existing SVD scale[107]. Three other scales[223-225] included a measure of atrophy. Atrophy can be considered a feature of CVD, and many studies have shown the presence and severity of atrophy does correlate with SVD[38, 107]. However, it is seen in a variety of other pathological and physiological conditions, notably neurodegenerative disease and normal ageing.

Table 1.9. Updated summary table: MRI indices of CVD

Study lead author	Characteristics	MRI markers of CVD	Outcome measure(s)
Chuang et al.[217]	62 vascular cognitive impairment and dementia patients.	7-point SVD ordinal scale (0-6) based on lacune, microbleed, and WMH severity.	Cross sectional association with grey and white matter volumes, blood pressure and carotid flow velocity.
Dickie et al.[222]	288 ppts in 3 cohorts of mild stroke, SLE and healthy volunteers.	"Brain health index" - combined neurodegenerative and neurovascular pathology. Divides voxels into healthy or pathological tissue	Association with Staals SVD score and cognitive performance.
Gomez-Choco et al.[218]	4424 stroke patients	3-level scale based on severity of WMH, presence of lacune, presence of microbleed.	Function and neurological recovery after acute ischaemic stroke (on discharge)
Jokinen et al.[224]	560 older adults with mild to moderate WMH	A combined z-score based on volumetric measures of WMH, lacunes, enlarged perivascular spaces, chronic cortical	Associations with longitudinal cognitive and functional outcomes.

		infarcts, and global and regional brain atrophy.	
Koton et al.[219]	907 stroke-free ppts	3-level scale of 'microvascular brain disease', based on severity of WMH and presence of lacunes.	Progression of microvascular brain disease and association with stroke
Van Slotten et al.[225]	1,949 ppts free of dementia and without baseline depressive symptoms	Score based on 5 features: high WMH volume, brain parenchyma volume, subcortical infarcts, cerebral microbleeds, and large PVS	Association between score and incident depressive symptoms.
Verwer at al.[220]	90 memory clinic patients with MRI-defined vascular brain injury	4-point SVD ordinal scale (0-3) based on WMH severity and presence of lacunes and/or microbleeds.	Associations with "physical performance"; gait speed, balance, and chair stand performance
Wang et al.[221]	436 older ppts	4-point "microvascular lesion load" ordinal scale (0-3) based on WMH severity, presence of lacunes and PVS severity.	Associations with cognitive decline and incident dementia over 9 years.

Abbreviations: ppts – participants; SVD – small vessel disease; CAA - cerebral microbleeds; PVS – dilated perivascular spaces; WMH – white matter hyperintensities.

Table 1.10 reports summary information of the 57 studies that have utilized indices reported in the published systematic review or in this update. The table has been divided by index. 43 papers used the Staals et al.[107] index, with a wide variety of associations examined. In addition to cognition and dementia, various neurological outcomes in at-risks groups were examined and associations with potential pathological mechanisms including BBB leakage[226, 227], various measures of hypertension and arterial stiffness[228, 229]. Six studies utilized modifications to the Staals scale[107], including adding an extra potential point for atrophy[230, 231], adding greater granularity to the contribution of constituent SVD markers[232], modifying the scale if there were missing markers, such as PVS[233] and making modifications to monitor longitudinal progression of SVD[234]. Five studies utilised the Charidimou et al. CAA-SVD scale[181] with one study attempting to simply the scale[235]. One study[236] used the Van Slotten et al. scale[225] and two paper utilized more than one scales[237, 238].

Table 1.10. Updated summary of studies utilizing an MRI index of CVD.

Study lead author	Characteristics	Neuroimaging index utilized	Outcome measure(s)
Amier et al.[239]	559 ppts with cardiovascular disease and controls	Staals	"Hypertensive exposure", operationalised through several heart MRI metrics
Arba et al.[226]	212 patients with acute ischemic stroke	Staals	Blood brain barrier leakage (assessed through perfusion-weighted images)
Banerjee et al.[240]	243 memory clinic patients	Staals	Cognitive domain, cortical atrophy and structural network measures.
Chen et al.[241]	202 patients with atherosclerotic cerebral small vessel disease	Staals	Association with deep medullary vein score
Chen et al.[242]	594 patients with various neurological symptoms	Staals	Association with lenticulostriate artery number
Del Brutto et al.[243]	331 ppts from rural population cohort	Staals	Cognition (MOCA)
Douven et al.[244]	188 stroke patients	Staals	Post-stroke depression and apathy

Du et al.[245]	127 SVD patients	Staals	Associations with structural brain network measures.
Feng et al.[246]	234 patients w. first-ever minor ischemic stroke or TIA	Staals	Vit D deficiency
Goldstein et al.[247]	449 patients with CVD	Staals	All-cause mortality
Hara et al.[248]	858 neurologically healthy adults	Staals	Associations with BMI, hypertension, smoking, and diabetes
Heinen et al.[249]	173 patients from memory clinic	Staals	Association with global brain network efficiency, and cognition
Jiang et al.[250]	57 patients with single small subcortical strokes	Staals	Progression of stroke within 72hrs from onset
Jiang et al.[251]	556 ppts from population study	Staals	Prospective cognitive change (MMSE) and incident dementia over 5 years
Kang et al.[252]	31 patients with periodic limb movements	Staals	Periodic limb movement index
Lau et al.[253]	1009 TIA /ischemic stroke patients	Staals	Associations with baseline and long-term premorbid blood pressure

Lau et al.[228]	587 patients with TIA or non-disabling ischemic stroke	Staals	Middle cerebral artery and internal cartoid artery pulsatility index (arterial stiffness)
Lau et al.[254]	959 patients with ischaemic stroke	Staals	Renal impairment
Li et al.[241]	314 ppts free of neurological disorders	Staals	Gait disturbance
Li et al.[255]	329 patients with ischaemic stroke	Staals	Associations with H-type hypertension (hypertensive patients with hyperhomocysteinemia)
Li et al.[227]	99 neurology outpatients without stroke	Staals	Associations with blood brain barrier integrity, assessed via DCE-MRI
Liang et al.[256]	743 acute ischemic stroke patients	Staals	Health-related Quality of Life
Liang et al.[257]	563 patients with acute ischemic stroke	Staals	Post-stroke depressive symptoms
Ling et al.[258]	41 CADASIL patients and 43 age- and sex-matched healthy controls.	Staals	Venous oxygen saturation - assessed by susceptibility weighted imaging and mapping
Lioutas et al.[259]	111 pts with spontaneous ICH	Staals	Associations with functional independence, ambulation and haematoma expansion

Liu et al.[260]	1080 patients from population-based study of TIA and ischemic stroke	Staals	Renal impairment
Loos et al.[261]	200 patients with minor lacunar or non-lacunar stroke	Staals	Gait disturbance
Lu et al.[262]	221 patients with ischaemic stroke	Staals	Extracranial artery stenosis
Onkenhout et al.[263]	132 memory clinic patients with SVD	Staals	Cerebral perfusion - assessed as global CBF on MRI
Riba-Llena et al.[229]	82 hypertensive individuals	Staals	Arterial stiffness (carotid–femoral pulse wave velocity)
Schoemaker et al.[264]	24 asymptomatic CADASIL subjects and 23 noncarrier	Staals	Executive function
Shi et al.[265]	20 CADASIL cases and 20 controls	Staals	Global cognition and specific cognitive domains
Shibata et al.[266]	71 patients with Parkinson's disease	Staals	Association with Parkinson's disease severity, vascular risk factors, motor phenotype and cognition
Shu et al.[267]	263 ischemic stroke/TIA patients	Staals	Retinal microvascular abnormalities

Suo et al.[268]	263 intracerebral haemorrhage patients	Staals	Association with haematoma expansion, within 72 hours of symptom onset
Suzuyama et al.[269]	1349 neurologically healthy ppts	Staals	Association with incident cerebro-cardiovascular events (mean 6.7 years)
Villain et al.[270]	113 patients with aneurysmal subarachnoid haemorrhage	Staals	Cerebral vasospasm, delayed cerebral ischemia and other clinical outcomes
Wei et al.[271]	207 patients with ischaemic stroke	Staals	Associations with haemorrhagic transformation
Woo et al.[272]	262 patients with first-ever acute cerebral infarction	Staals	Association of plasma Klotho concentration with the presence, burden and progression of SVD
Yakushiji et al.[273]	1,451 neurologically healthy adults	Staals	Cross sectional association with vascular risk factors, cognition (MMSE) and cerebral atrophy
Yilmaz et al.[274]	1651 from population cohort.	Staals	Association of the CSVD score with risk of stroke, dementia, and mortality
Yu et al.[275]	130 subjects with differing burden of SVD	Staals	Global and regional cerebral perfusion
Zhang et al.[276]	904 ppts from rural population cohort	Staals	Associations with extracranial artery indices - brachial-ankle pulse wave velocity, the ankle-

			brachial index, the carotid intima-media thickness, and carotid plaque
Amin Al Olama et al.[233]	1165 ppts from 3 longitudinal cohorts, with varying degree of SVD pathology	Modified Staals - no PVS, score 0-3	Association with incident dementia
Appleton et al.[277]	4,011 patients with acute stroke	Modified Staals for CT - only leukokariosis and lacunes.	Functional change (modified Rankin Scale score) at day 90
Fan et al.[230]	140 inpatients with CVD	Modified Staals - extra potential point for atrophy	Association with 24 hr blood pressure variability
Jimenez-Balado et al.[234]	33 ppts with a median age of 65 years, stroke free	Modified Staals scale, to monitor progression over time.	Association with ambulatory blood pressure
Xu et al.[232]	108 patients with primary intracerebral haemorrhage	Modified Staals - two variants adding greater granularity to rating of	Renal function

		WMH, CMB and PVS (new total score of 0-10)	
Yang et al.[231]	251 neurology inpatients	Modified Staals - extra potential point for atrophy	Cross sectional association with SBP levels and variability
Boulouis et al.[278]	229 CAA patients with ICH	Charidimou- CAA-SVD scale	Prediction of ICH recurrence
Li et al.[279]	Eighty patients with probable CAA	Charidimou CAA scale	Presence of cortical microinfarcts
Valenti et al.[280]	73 pts with CAA	Charidimou- CAA-SVD scale	Association with global network efficiency (diffusion tractography)
Xiong et al.[281]	158 patients from the Stroke Service or memory clinic with probable CAA	Charidimou- CAA-SVD scale	Conversion to dementia at 1 and 5 years.
Yilmaz et al.[235]	1622 stroke-free and dementia-free ppts of population-based study.	Modified 'simplified' version of Charidimou CAA scale - the presence	Associations with cognitive impairment, stroke, dementia, and mortality

		of strictly lobar CMB, cSS, CSO-PVS, and WMH.	
Rensma et al.[236]	2,135 individuals without dementia and baseline depression	Van Slotten	Whether SVD mediates the association between type 2 diabetes and higher depressive symptoms
Banerjee et al.[237]	114 ischaemic cardioembolic stroke or TIA patients with AF	2 scales- Charidimou-CAA-SVD scale and Staals	Cognitive change at 12 months (MOCA)
Pasi et al.[238]	612 ICH survivors	3 scales - Charidimou, Staals and Van Slotten	Association of longitudinal cognitive decline and incident dementia

Staals: Staals et al[107].; Charidimou: Charidimou et al[181].; Van Slotten: Van Slotten et al.[225]

Abbreviations: ppts – participants; SVD – small vessel disease; CAA - cerebral amyloid angiopathy; TIA – transient ischemic attack; PVS – dilated perivascular spaces; WMH – white matter hyperintensities; CBF – cerebral blood flow; MMSE- mini-mental state examination.

Discussion:

There appears to be increasing research interest in the area of CVD indices, with 65 papers published in the period of the updated review, compared to just 25 papers that were discussed in the original review, dating from database inception to 2017.

Despite this interest however, there have been few novel approaches to the construction of new indices. The limitations of the eight new scales are similar to the limitations discussed in the original publication. These include the fact that there are limited number of MRI markers included in the scale and that these have generally been confined to lesions seen on structural sequences. Markers from non-structural sequences including DTI, perfusion techniques or network analysis have not been included. The indices may therefore be missing out on capturing some of the variability of CVD. One exception was the Brain Health Index[222], which used a voxel-based Gaussian mixture model cluster analysis of T1, T2, T2*, and FLAIR sequences to define pathological tissue. This approach is automated and therefore can be quicker than manual assessment and also takes a whole brain approach. Limitations include that it does not discriminate by lesion type, that it cannot differentiate between neurodegenerative and vascular pathologies and it is difficult to implement clinically.

Zeestraten et al.[282] examined which combination of MRI markers was best associated with longitudinal cognitive decline and incident dementia. Although not operationalised as an index, they did find that a model including change in WMH lesion load and a DTI metric, normalized peak height of mean diffusivity histogram distribution best predicted progression to dementia.

1.8 Aims and hypotheses

The overarching aim of this PhD thesis was the exploration of new and existing neuroimaging methods of quantification of CVD burden. Specifically:

1. To explore the contribution of two, under-researched but common individual MRI markers, namely CMB and PVS.
2. To develop and validate a novel CVD index, comprised of several empirically weighted constituent MRI markers.

The primary hypothesis was that a multi-modal index would better capture the variability of CVD burden than any individual marker. Global cognition was chosen as the dependent variable representing CVD burden.

Secondary hypotheses were:

1. That the developed CVD index would show a stronger association with Global Cognition than the best-established existing index[184], developed from structural imaging.
2. That the performance of the CVD index would be improved by the inclusion of a DTI measure(s).

Chapter 2: The relationship of cerebral microbleeds to cognition and incident dementia in non-demented older individuals

2.1 Overview

Chapter 2 examines the cognitive sequalae of CMB, a marker of SVD that results from small perivascular hemosiderin deposits, following microscopic bleeds. Within the 12 neuroimaging indices of CVD identified in the original systematic review and update, they were included in seven, with only WMH and lacunes being included more often. The scaling of the CMB variable has been performed in a variety of ways, including global severity, severity of deep CMB and consideration of just multiple (≥1) CMB. Individual studies have reported inconsistent associations with longitudinal cognitive decline and incident dementia, which may be a result of regional differences[71, 283].

Chapter 2 aims to determine whether the presence of CMB, measured both globally and in specific cerebral regions, was associated with cognitive impairment cross-sectionally, and with cognitive decline and incident dementia over four years of follow up.

Susceptibility weighted imaging (SWI) was used to assess CMB in 302 older participants of the population based longitudinal Sydney Memory and Ageing study. CMBs were recorded throughout the brain and divided into lobar and deep regions for analysis, thought to represent two different (albeit potentially overlapping) pathologies. Specific cognitive domains as well as Global Cognition were examined, with linear mixed models used to model longitudinal change.

2.2 Publication: Paradise M, Seruga A, Crawford JD, et al. The relationship of cerebral microbleeds to cognition and incident dementia in non-demented older individuals. Brain Imaging Behav 2019;13:750-761.

Erratum: Table 3.

Table 3 in the published manuscript (see Appendix) contains a typographical error only identified after publication. In the final column of the table, where the association between whole brain multiple CMB and executive function is presented (Model 3), the cell is displayed as *−0.41 (−0.78 to 0.03)*.* The 95% upper confidence interval estimate should be preceded by a – symbol, to correctly read: *−0.41 (−0.78 to -0.03)*.* The correct results are displayed in the body of the thesis below.

The relationship of cerebral microbleeds to cognition and incident dementia in non-demented older individuals

Abstract

Background: Cerebral microbleeds (CMB), suspected markers of hemorrhage-prone microangiopathy, are common in patients with cerebrovascular disease and in those with cognitive impairment. Their longitudinal relationship with cognitive decline and incident dementia in non-demented community-dwelling older individuals has been insufficiently examined.

Methods: 302 adults aged 70-90 participating in the population-based Sydney Memory and Ageing Study underwent a susceptibility-weighted imaging (SWI) MRI sequence. The relationship of CMB with performance on neuropsychological tests was examined both cross-sectionally and longitudinally, over a mean of 4 years. The association with cases of incident dementia during this period was also examined.

Results: The prevalence of CMB was 20%. In cross-sectional analysis, after adjusting for demographics and vascular risk factors, there was a significant association between the presence of CMB and poorer executive function. CMB were not associated with global cognition or other cognitive domains. On longitudinal analysis, after adjusting for demographics and vascular risk factors, there was a greater decline in visuospatial ability in those with CMB compared to those without. The presence of CMB was not associated with increased progression to dementia.

Conclusions: CMB are associated with impairments in specific cognitive domains: executive function and decline in visuospatial ability, independent of other markers of CVD including white matter hyperintensities. This suggests a direct contribution of CMB to cognitive impairment although no significant difference in incident dementia rates was observed.

Background

Vascular pathology is associated with cognitive impairment and dementia, and several markers of cerebral small vessel disease (SVD) can be visualized using magnetic resonance imaging (MRI). In addition to white matter hyperintensities (WMH) and lacunes, cerebral microbleeds (CMB) have been recognized as a marker of SVD. CMB are visualized as punctate hypointense lesions on paramagnetic-sensitive MRI sequences, in particular susceptibility weighted imaging (SWI), and can be found throughout the cerebral lobes, basal ganglia, cerebellum and brainstem [68]. They correspond to small perivascular haemosiderin deposits and represent breakdown products from prior microscopic hemorrhages [65, 284].

CMB are found in both cognitively normal and impaired individuals, and their prevalence increases with age from 7% at age 45-50 years to 36% at 80 years and older [66]. While their prevalence is highest in vascular dementia (65-85%), CMB are also frequently found in Alzheimer's disease (AD) (18-32%) and mild cognitive impairment (MCI) (20-43%) [67]. The distribution of CMB in the brain differs according to underlying pathology. CMB in the subcortical and deep regions are thought to be associated with atherosclerotic/hypertensive small vessel disease and in contrast, CMB in lobar regions are more likely associated with cerebral amyloid angiopathy (CAA) [68, 69].

Once thought to be clinically silent, the majority of cross-sectional studies have reported an association of CMB with impaired executive function, attention and processing speed and, in some studies, global cognition [285-287]. The location and number of CMB within the brain are also pertinent, with several large studies [69, 288] and a recent meta-analysis [287] suggesting that cognitive impairment is seen in the presence of multiple CMB. Moreover, CMB located in the lobar and deep regions, but not infratentorial lesions [287, 289], have an association with cognitive impairment. The studies examining the longitudinal impact of CMB on cognitive decline and incident dementia have reported inconsistent results [70, 288, 290-296]. There are multiple reasons for the inconsistent findings: lack of common consensus criteria for CMB; diverse populations; different imaging parameters; and diverse use of cognitive measurement.

In the context of this inconsistency, and the scarcity of longitudinal studies, the aims of this study were to determine whether the presence of CMB, measured both globally and in specific cerebral regions, was associated with cognitive impairment cross-sectionally, and with cognitive decline and incident dementia over four years of follow-up.

Methods

Subjects

Participants were drawn from the population based longitudinal Sydney Memory and Ageing study (MAS) [151], an ongoing study which began in 2005 and focuses on cognitive decline in the community-dwelling elderly. Subjects were aged 70-90 years, living in the community and able to complete their assessments in English. Exclusion criteria were diagnosis of dementia or other psychiatric or central nervous system disorder at wave 2. There have been four waves of this study, two years apart. At each wave, participants underwent an MRI scan, comprehensive neuropsychological assessment, medical examination, blood collection, and *APOE* genotyping (wave 1). For this study, we used data from wave 2, when SWI was introduced to the MRI protocol. Written consent was obtained from all participants. The methodological details and ethics approval of the study have been previously published [151].

Radiological examination

MRI was performed on a Philips 3T Achieva Quasar Dual scanner (Philips Medical Systems, Best, Netherlands). The parameters for the SWI sequence were repetition time (TR)=25.33 ms, time to echo (TE)=40.33 ms; slice thickness=1.1, of field of view (FOV)=240×132×215 with overlap of 0.55 mm (over-contiguous) with no gap between slices producing a spatial resolution of 0.536×0.536×0.50 mm^3/voxel. A 3D T1-weighted sequence (1×1×1 mm3, TR/TE=6.39/2.9 ms) and a T2-weighted fluid attenuation inversion recovery (FLAIR) sequence (TR/TE/TI=10000/110/2800 ms; thickness 3.5 mm; 0.898×0.898 mm2) were also performed. Total WMH volume was assessed with automated methods using FLAIR and T1-weighted images, and adjusted for total intra-cranial volume [40].

All images were co-registered using Statistical Parametric Mapping version 5 (SPM5) (Wellcome Department of Cognitive Neurology, London, UK, 1999) using T1 images as reference and SWI images as source. CMB were defined as round hypointense foci <10 mm in diameter seen on SWI, at least half surrounded by brain parenchyma and distinct from potential mimics such as iron and calcium deposits, vessel flow voids and bone[68]. Symmetrical hypointensities in the globus pallidus likely representing calcium or non-hemorrhagic iron deposition were excluded. Hypointensities within the subarachnoid space were deemed to be pial blood vessels. The co-registered T1 images were used to determine precise anatomical localization and help exclude sulcal flow voids.

All images were analyzed using MRIcron version 15 (www.nitrc.org/projects/mricron/). For each image, the location, number and size (based on maximum diameter) of any identified CMB were recorded. The counted anatomical locations included the frontal, temporal, parietal and occipital lobes, the basal ganglia including the caudate, putamen and globus pallidus, the thalamus, brainstem and cerebellum. Any uncertain lesions were reviewed with an experienced neuroradiologist (JC). Twenty scans were excluded due to excessive motion or susceptibility artefacts. Participants whose scans were excluded did not significantly differ from the included participants in age, sex, blood pressure, education or cognitive impairment. All images were analyzed blind to clinical data.

Inter-observer reliability on 10 randomly selected scans (CMB absent vs. present) between the primary rater (AS) with an experienced neuroradiologist (JC), was Cohen's κ=0.615, corresponding to 'good' agreement. Intra-observer reliability on 20 randomly selected scans rated 2 months apart was Cohen's κ=1.0, corresponding to 'very good' agreement.

Neuropsychological assessment

Neuropsychological assessment, administered by trained research psychologists, consisted of a battery of tests grouped into cognitive domains: attention and processing speed (Digit Symbol-Coding [297], and Trail Making Test (TMT) A [298]), memory (Logical Memory [299], Rey Auditory Verbal Learning Test (RAVLT), [298] and Benton Visual Retention Test [300]), language (Boston Naming Test – 30 items [301], Semantic Fluency (Animals) [298]), visuospatial (Block Design [302]), and executive function (Controlled Oral Word Association Test (FAS) [298] and Trail Making Test (TMT) B [298]). Raw test scores were transformed to z-scores using the baseline mean and SD values of a healthy reference subsample (n=723 MAS participants). Domain scores were calculated by averaging the z-scores of component tests (except for visuo-spatial which was represented by a single test) and a global cognition score was calculated by averaging the domain scores. These composite scores were standardized so that the healthy reference group had means and SDs of 0 and 1 respectively.

Diagnostic classification was based on a multidisciplinary consensus panel consisting of old age psychiatrists, neuropsychiatrists and neuropsychologists using established criteria for MCI [303] and DSM-IV [304]/DSM-5 [305] criteria for dementia conducted at each wave [151].

Assessment of covariates

Data on potential risk factors for CMB in the MAS study were collected through face-to-face assessments by trained research psychologists. Participants gave a blood sample for cholesterol, lipoprotein levels and genetic analysis including apolipoprotein gene (*APOE*) status. Hypertension was defined as a blood pressure of ≥140/90 mm/Hg taken as the mean of two seated readings or if the participant was ever diagnosed as having hypertension by their doctor. Diabetic status was determined by a prior medical diagnosis or a fasting blood glucose value >7 mmol/L.

Cardiovascular risk was computed based on the research of the Framingham Stroke Study [306]. Specifically, our variable was based on the 10-year risk prediction of general cardiovascular disease (http://www.framinghamheartstudy.org/risk-

functions/cardiovascular-disease/index.php), using the following: sex; current smoking status (self-reported); diabetic status; systolic blood pressure; total cholesterol; high-density lipoprotein (HDL); currently on antihypertensive medication. When blood analyses were unavailable, Body Mass Index (BMI) was used instead of cholesterol and HDL data (as per Framingham protocol). See D'Agostino et al. [306] for the weighting of the variables in the index. We excluded age from the score calculator as the range of ages of 70 years plus in our sample would have produced a ceiling effect (or at least very little variation) in the calculated risk score. Age was therefore included as a separate covariate in the analysis.

Statistical analysis

First, the numbers of CMB in the lobar and deep regions of the brain, as well as the whole brain (lobar, deep or infratentorial regions), were calculated. The lobar brain region was defined as comprising the frontal, temporal, parietal and occipital lobes, and the deep region comprising the basal ganglia and thalamus. A subgroup was created with CMB *only* in the lobar regions (strictly lobar distribution). Individuals with CMB in more than one regions were considered to have a mixed distribution. A strictly deep group was not created as there were insufficient numbers for meaningful analysis. An infratentorial group was not created as several studies reported that CMB in this location were not associated with cognitive impairment [287, 307]. Two variables were formed to record CMB numbers: a binary variable of CMB present versus no CMB and a categorical three-level variable of 0 CMB, 1 CMB and multiple ($\geq$ 2) CMB. The effects of multiple ($\geq$ 2) CMB were explored as multiple microbleeds have been more consistently associated with cognitive deficits than a single CMB [109] and the rating of one CMB has been reported to be less reliable [308, 309].

Descriptive statistics for sample characteristics at baseline were presented for subsamples with and without CMB, and also for the subsample with multiple microbleeds. To examine differences in demographic and medical characteristics between those with no CMB and the other two subsamples, independent samples t-tests were used for continuous variables, Mann-Whitney U tests were used for non-

normal continuous variables (absolute value of skewness >1) and Pearson's chi-square tests or Fisher's exact test for categorical variables.

Distributions of the dependent variables were inspected for normality and extreme values. Where necessary, distributions were transformed to more closely approximate the normal distribution (absolute value of skewness less than one). Also, to minimize the influence of extreme values on statistical outcomes, scores were Winsorized where necessary so that upper and lower values were reduced to three standard deviations above or below the mean.

A series of ANCOVAs (Analysis of Covariance) were used to examine differences in domain and global cognition measures at wave 2 between first, the no CMB group and the CMB present group and second, between the no CMB and the multiple CMB group. This was done for the CMB in the whole brain and separately for those with a strictly lobar distribution. Analyses were performed with covariates age, sex and education (Model 1), repeated with the additional covariates of cardiovascular risk score and *APOE-ε4* (Model 2) and finally with the addition of markers of cerebral SVD; WMH volume and presence of lacunes (Model 3).

For the longitudinal analysis, linear mixed-models (LMMs) were used to examine the association of the two CMB variables with changes in global and domain scores across the three- time-points; waves 2, 3 and 4. In these analyses, wave was treated as a repeated measures factor, the CMB variables, between-subject factors, and interactions between the CMB and wave were included in the equations. A random intercept term was also included. Estimated means were obtained for each of the time points and charted. Custom contrasts were then used to examine differences in cognitive changes (linear trend) between the CMB groups across the three waves. As for the cross-sectional analyses, the longitudinal analyses were performed with covariates age, sex and education (Model 1), repeated with the additional covariates cardiovascular risk score, *APOE-ε4* (Model 2) and with the additional markers of cerebral SVD; WMH volume and presence of lacunes (Model 3). Analyses were

performed both for CMB in the in the whole brain and separately for those with a strictly lobar distribution.

Logistic regression models were used to estimate the odds ratio (95% Confidence Interval) of developing dementia between waves 2 and 4 on the basis of the two CMB factors described above; the binary categorical variable and the 3-level factor (≥2 CMB vs none), across the whole brain and then for those with a strictly lobar distribution of CMB. These analyses were performed with the same covariates as the LMM analysis above (Models 1 to 3). Due to potential attrition bias, a conservative sensitivity analysis was performed, assuming that the proportion of non-completers at wave 4 was the same as the proportion of completers have dementia. This was done by re-running the logistic regression, randomly re-assigning non-completers to diagnostic categories (dementia vs. no dementia) to make up the correct assumed proportions.

All analyses were performed using the SPSS statistical package (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp).

Results

Of the 302 participants, 60 (20%) had at least one CMB. Of these 41 (68%) had CMB only in a lobar distribution; 4 (7%) had CMB only in a deep distribution and 15 (25%) had CMB in a mixed distribution and/or the infratentorial area. Half had only one CMB (n = 30, 50%) and half had more than one CMB (n = 30, 50%).

Baseline characteristics of the study population are reported in Table 2.1. Total WMH volume significantly differed between CMB present and CMB absent groups ($U =$ 8667.5, $z = 2.91$, $p = 0.004$) and between the multiple CMB and CMB absent groups ($U = 4571.5$, $z = 2.60$, $p = 0.009$). No other demographic or medical characteristics significantly differed between the groups.

Table 2.1: Baseline characteristics of the study population (n=302) and associations with cortical microbleeds (CMB).

	CMB absent (Reference)	CMB present (≥1)	Multiple CMB (≥2)
Total	242	60	30
Age, years, mean ± SD	79.4 ± 4.4	79.8 ± 4.6	79.1 ± 4.7
Men, number (%)	110 (46)	31 (52)	14 (47)
Education, years ± SD	11.9 ± 3.6	11.7 ±3.4	12.1 ± 3.6
MMSE, mean ± SD	28.4 ± 1.4	28.1 ± 1.5	28.1 ± 1.4
MCI, number (%)	86 (36)	20 (33)	11 (37)
BMI, mean ± SD[a]	26.7 ± 4.1	26.4 ± 4.0	25.2 ± 3.0
Smoker (in last month), number (%)[b]	9 (5)	4 (8)	3 (12)
Hypertension, n (%)[c]	160 (66)	39 (65)	18 (60)
Diabetes, number (%)	46 (19)	9 (15)	6 (20)
Cardiovascular Disease Risk Factor Score, mean ± SD[d]	3.6 ± 3.2	4.4 ± 3.8	3.8 ± 4.4
APOE-ε4 carrier, n (%)[e]	61 (25)	14 (23)	8 (27)
Total WMH volume mm^3, mean ± SD[f]	14,565 ± 12,268	22,763 ± 22,018**	26,090 ± 24,306**
Number of lacunes, mean ± SD[g]	0.11 ± 0.45	0.15 ± 0.55	0.20 ± 0.55
Presence of lacunes, n (%)[h]	20 (8)	5 (9)	4(13)

Abbreviations: SD, Standard Deviation; MMSE, Mini Mental State Examination; MCI, Mild Cognitive Impairment; BMI, Body Mass Index; WMH, White Matter Hyperintensity.

Data are missing for [a] 7 [b] 55 [c] 1 [d] 11 [e] 2 [f] 7 [g] 2 [h] 2 participants respectively.

*p<0.05; ** p<0.01; *** p<0.001

p-values are for comparisons with the CMB absent group.

Cross-sectional results: When adjusted for age, sex and education, there was no association between either the presence of CMB or multiple CMB (vs no CMB) anywhere in the brain (whole brain) and global cognition or the domains attention and processing speed, language, visuospatial ability and memory (Table 2.2). There were significant associations between the presence of CMB and executive dysfunction, for both the presence of CMB (cf. no CMB; mean difference in z-score -0.35; 95%CI -0.63 to -0.07, p=0.015) and when examining participants with multiple (≥2) microbleeds (cf. no CMB; mean difference in z-score -0.53; 95%CI -0.90 to -0.17, p=0.004).

These associations remained significant when further adjusted for the presence of cardiovascular risk score and *APOE-ε4* status (Model 2) (Table 2.3). Moreover, when additionally adjusted for the markers of SVD; WMH volume and the presence of lacunes (Model 3), those individuals with multiple microbleeds retained a significant association with executive function. (cf. no CMB; mean difference in z-score -0.41; 95%CI -0.78 to -0.03, p=0.035).

Table 2.2. Cross-sectional relationship of cerebral microbleeds (CMB) with cognition at Wave 2; expressed as differences in neuropsychological domain scores from group with no CMB, (95% CI) (N=302, number with CMB=60).

Type and No. of Microbleeds	No. of Participants	Global Cognition	Attention and Processing Speed	Executive	Language	Visuospatial	Memory
				Cognitive Domain			
				Whole Brain			
CMB present	60	-0.18 (-0.45 to 0.08)	-0.11 (-0.39 to 0.18)	-0.35* (-0.63 to -0.07)	0.02 (-0.24 to 0.27)	-0.15 (-0.43 to 0.14)	-0.15 (-0.40 to 0.11)
Multiple CMB	30	-0.31 (-0.78 to 0.17)	-0.30 (-0.67 to 0.07)	-0.53** (-0.90 to -0.17)	0.02 (-0.32 to 0.37)	-0.24 (-0.62 to 0.13)	-0.22 (-0.57 to 0.12)
				Strictly Lobar Distribution			
CMB present	41	-0.14 (-0.45 to 0.17)	-0.02 (-0.35 to 0.31)	-0.24 (-0.56 to 0.09)	0.02 (-0.28 to 0.32)	-0.22 (-0.56 to 0.11)	-0.07 (-0.37 to 0.23)
Multiple CMB	15	-0.26 (-0.73 to 0.22)	-0.23 (-0.74 to 0.27)	-0.43 (-0.92 to 0.07)	0.08 (-0.40 to 0.55)	-0.29 (-0.81 to 0.23)	-0.10 (-0.57 to 0.37)

Test-wise *p<0.05; **p<0.01

Model 1: All analyses adjusted for age, sex and educational level.

Missing data: Global Cognition 4 participants, Attention and Processing Speed 8 participants, Language 1 participant, Executive function 12 participants, Visuospatial 5 participants.

Table 2.3. Cross-sectional relationship of cerebral microbleeds (CMB) with executive function, adjusted for vascular risk factors and markers of SVD; expressed as differences in Neuropsychological Domain scores from CMB absent group, (95% CI) (N=278, number with CMB=52).

Type and No. of Microbleeds	No. of Participants	Executive function	
		Model 2	Model 3
Whole Brain			
CMB present	52	-0.33 (-0.62 to -0.04)*	-0.26 (-0.55 to 0.04)
Multiple CMB	29	-0.51 (-0.88 to -0.14)**	-0.41 (-0.78 to -0.03)*
Strictly Lobar Distribution			
CMB present	36	-0.22 (-0.55 to 0.12)	-0.20 (-0.53 to 0.14)
Multiple CMB	15	-0.41 (-0.91 to 0.09)	-0.40 (-0.90 to 0.10)

Test-wise *p<0.05; **p<0.01

Model 2 - adjusted for age, sex, educational level and Cardiovascular risk score, APOE-4 status

Model 3 - adjusted for age, sex, educational level and Cardiovascular risk score, APOE-4 status, WMH and lacunes.

Longitudinal results: Results for Model 1 (adjusted for age, sex and education) are presented in Table 2.4 and displayed in Figure 2.1, for CMB across the whole brain. The mean duration between wave 2 and wave 4 was 47.6 months (SD 1.9; range 40 – 57). Thirty-four individuals (11%) did not attend for wave 4 cognitive testing. Compared to those who attended wave 4, at baseline (wave 2) these 34 participants were significantly older, were more likely to have received a diagnosis of MCI and had worse cognition (MMSE and significantly reduced impairments in global cognition, executive function and memory and a trend towards worse attention and processing speed). They did not differ significantly in their gender, cardiovascular risk score, education, WMH volume or proportion with lacunes.

Table 2.4. Longitudinal relationship of whole brain cerebral microbleeds (CMB) with changes in cognition; expressed as estimated marginal means, adjusted for age, sex and education (Model 1; N=302[a], number with CMB=60).

Cognitive domain		Estimated marginal z-score mean [a]			Interaction contrast representing difference between CMB groups in change from W2 to W4		
		Wave 2	Wave 3	Wave 4	Value (CI)	t	p-value
Global Cognition	CMB absent	0.07	-0.07	-0.34			
	CMB present	-0.11	-0.25	-0.61	0.09 (-0.08 - 0.26)	1.05	0.294
	CMB absent	0.07	-0.07	-0.34			
	Multiple CMB (≥ 2)	-0.26	-0.32	-0.68	0.01 (-0.22 - 0.24)	0.10	0.924
Attention and Processing speed	CMB absent	0.03	-0.15	-0.38			
	CMB present	-0.07	-0.40	-0.62	0.14 (-0.09 - 0.38)	1.21	0.227
	CMB absent	0.03	-0.15	-0.38			
	Multiple CMB (≥ 2)	-0.26	-0.45	-0.58	-0.10 (-0.41 - 0.21)	-0.61	0.545
Executive function	CMB absent	0.03	-0.12	-0.31			
	CMB present	-0.33	-0.38	-0.52	-0.15 (-0.35 - 0.05)	-1.52	0.130

	CMB absent	0.03	-0.12	-0.31			
	Multiple CMB (≥ 2)	-0.50	-0.36	-0.58	-0.26 (-0.52 – 0.00)	-1.99	0.047
Language	CMB absent	-0.07	-0.09	-0.26			
	CMB present	-0.05	-0.16	-0.29	0.06 (-0.14 - 0.26)	0.56	0.575
	CMB absent	-0.07	-0.09	-0.26			
	Multiple CMB (≥ 2)	-0.05	-0.17	-0.35	0.12 (-0.15 - 0.39)	0.86	0.392
Visuospatial	CMB absent	0.24	0.14	-0.10			
	CMB present	0.10	0.01	-0.50	0.26 (0.03 - 0.48)	2.27	0.024
	CMB absent	0.24	0.14	-0.10			
	Multiple CMB (≥ 2)	0.00	-0.07	-0.62	0.28 (-0.02 - 0.58)	1.82	0.069
Memory	CMB absent	0.02	-0.02	-0.21			
	CMB present	-0.13	-0.16	-0.30	-0.05 (-0.26 - 0.15)	-0.53	0.597
	CMB absent	0.02	-0.02	-0.21			
	Multiple CMB (≥ 2)	-0.21	-0.28	-0.30	-0.14 (-0.41 - 0.13)	-1.03	0.304

[a] - at wave 2.

Figure 2.1. Estimated mean z-scores for global and specific cognitive domains, across waves 2, 3 and 4 (whole brain). Means adjusted for age, sex and education.
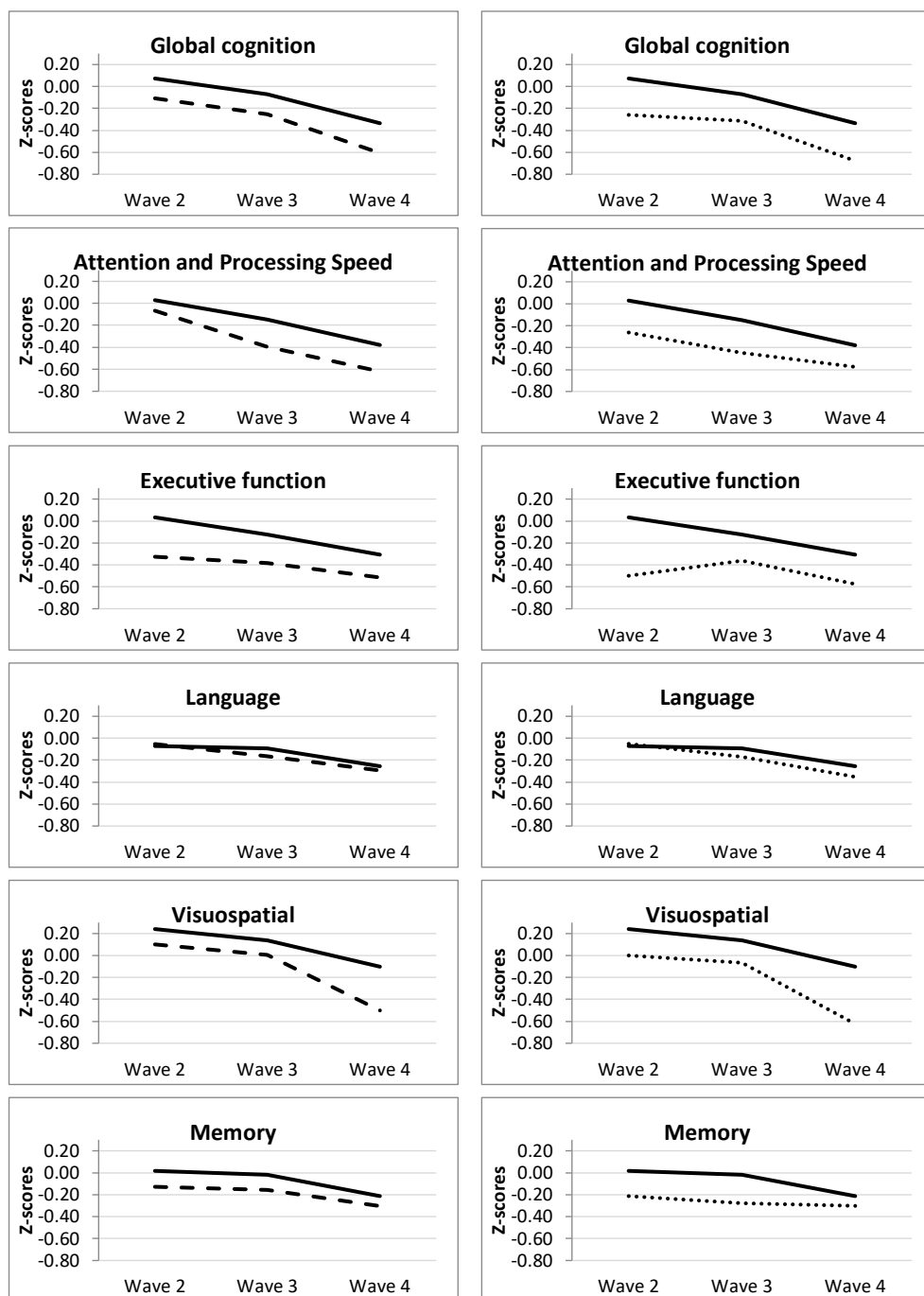
Legend: No CMB —————— No CMB ——————

CMB present – – – – Multiple CMB ·············

There was a strong association between CMB and longitudinal change in visuospatial ability. Participants with any CMB (whole brain) had a significantly faster decline (linear trend) in visuospatial function score compared to those with no CMB (Contrast estimate 0.26; SE 0.11; t(531.92) = 2.27, p = 0.024), adjusted for age, gender and education. This association remained significant in Model 3, after further adjusting for presence of cardiovascular risk, *APOE-ε4* status, WMH and lacunes (Contrast estimate 0.28; SE 0.12; t(496.61) = 2.41, p = 0.016). These associations remained significant when examining only those participants with a strictly lobar distribution of microbleeds. These associations, although similar in magnitude, were no longer statistically significant when those with multiple whole brain CMB were analyzed, with only a non-significant trend toward significance (Contrast estimate 0.28; SE 0.15; t(532.63) = 1.82, p = 0.069). Please see Supplemental Table 2.6 for full results.

There was an association between CMB and longitudinal change in executive function. Participants with multiple CMB (whole brain) had a significantly *slower* decline (linear trend) in executive function score compared to those with no CMB (Contrast estimate -0.26; SE 0.13; t(511.22) = -1.99, p = 0.047, adjusted for age, gender and education. This association no longer remained significant after further adjusting for the presence of cardiovascular risk, WMH and lacunes (Model 3). There was not an association between executive function and participants with any CMB across the whole brain or those with CMB in a strictly lobar distribution. Please see Supplemental Table 2.7 for full results.

Whole brain CMB were not associated with decline in global cognition, attention and processing speed, language or memory.

Dementia diagnosis: Table 2.5 reports the association of CMB with incident dementia. Nine percent of those without CMB had dementia at wave 4, compared to 15% of those with a CMB. This was not a significant result however; (presence of any CMB cf. CMB OR 1.77; 95%CI 0.70–4.48, p=0.229), when adjusted for age, sex and education. These results did not change when only the groups with multiple microbleeds or those with a strictly lobar distribution of CMB were examined. Nor did the results significantly change with the sensitivity analysis (presence of any CMB cf. no CMB OR 1.54; 95%CI 0.65–3.65, p=0.322).

Table 2.5. Association of cerebral microbleeds (CMB) with incident dementia at wave 4.

	Raw probabilities P (n/N)			Test for difference in ORs at wave 4		
	Wave 2 N=286	Wave 3 N=268	Wave 4 N=267	OR	CI	p-value
Whole brain CMB						
CMB absent	0.00 (0/229)	0.06 (13/215)	0.09 (20/215)			
CMB present	0.00 (0/57)	0.04 (2/53)	0.15 (8/52)	1.77	0.70–4.48	0.229
CMB absent	0.00 (0/229)	0.06 (13/215)	0.09 (20/215)			
Multiple CMB (≥ 2)	0.00 (0/27)	0.07 (2/27)	0.12 (3/26)	1.42	0.37–5.45	0.608
Strictly lobar distribution of CMB						
CMB absent	0.00 (0/229)	0.06 (13/215)	0.09 (20/215)			
CMB present	0.00 (0/38)	0.03 (1/37)	0.16 (6/37)	1.90	0.67 – 5.36	0.228
CMB absent	0.00 (0/229)	0.06 (13/215)	0.09 (20/215)			
Multiple CMB (≥ 2)	0.00 (0/14)	0.07 (1/14)	0.08 (1/13)	0.91	0.10- 7.95	0.932

Discussion

In this population based study of non-demented older adults, we found a CMB prevalence of 20%. On cross-sectional analysis, after adjusting for demographics and cardiovascular risk factors, participants with CMB were more likely to have impairments in executive function compared to those without CMB. When examined longitudinally over four years this association was not sustained. In contrast to the cross-sectional results, on longitudinal analysis, those with CMB were more likely to have a greater decline in visuospatial function that those without CMB. Those with CMB at baseline were not more likely to have or develop dementia during the follow up period.

The literature on the association of CMB with cognitive impairment is complex and at times contradictory. Consistent with our cross sectional results however, the majority of studies that found an association between CMB and cognitive impairment (and a recent meta-analysis [283]) reported deficits in executive function [67, 70, 310-312], with several finding that executive function was the only cognitive domain associated with CMB [310, 313]. The association between CMB and greater decline in visuospatial function over time is less frequently reported and most studies did not investigate this cognitive domain specifically. Several Asian studies however, did find an association of CMB with visuospatial decline in cohorts including subcortical vascular dementia [314], cognitively impaired elders [315] and community dwelling aged [291].

Executive function is one of the most commonly reported domains to be impacted by SVD generally, supporting the argument that CMB may be a proxy for generalized vascular disease and its subsequent sequelae. Increased numbers of CMB are often in regions thought to be the neuroanatomical substrate of these cognitive domains, i.e., the frontal and basal ganglia, with the hypothesis that lesions in these regions disrupt critical frontal-subcortical circuits [312] producing specific cognitive impairments [316]. We found that individuals with a strictly lobar distribution of CMB did not have impaired executive function. This may suggest the association is driven by deep cerebral SVD, or represent the limitations of a smaller sub-sample size. Participants with CMB had

worse executive function at wave 2, but not a steeper decline in executive function with time, which may be explained by the particularly poor executive function results of the CMB group in wave 2. This affected the slope of the linear trend (Figure 1) and meant not only did this group not decline faster, but paradoxically that in some analyses (those with multiple microbleeds across the whole brain) participants with CMB had an *improvement* in executive function over time (compared to those without CMB). We assessed visuospatial ability through Block Design, but this test incorporates a range of other cognitive abilities, including aspects of executive function and processing speed which are a particularly important contributor to performance above age 75 [317, 318].   We categorized the tests into domains a priori according to the principal cognitive function that they represented according to convention and psychological theory. However, neuropsychological tests are multifactorial in structure and even though a test may be designed to focus on one aspect of cognition, test performance involves multiple cognitive processes [319]. This overlap and lack of clear delineation between different cognitive domains is one of the reasons for the complex and often contradictory results in the published literature. Indeed, when a principal component analysis was previously performed on the MAS data (unpublished) the first principal component comprised attention/processing speed, executive function and visuospatial factors. Interestingly Chung et al. [291] reported that strictly lobar CMB were associated with impairments in a visuospatial executive function, but not with a verbal executive function domain.

There was a strong association between CMB presence and increased WMH volume. WMH are a well-established marker of SVD and our finding that CMB is associated with cognitive impairment independent of WMH volume supports the thesis that CMB produce cognitive impairment through direct mechanisms as well as being a general marker of overall cerebral vascular pathology. Several other studies have now shown that CMB are associated with cognitive impairment independent of WMH volume [70, 311, 320]. Supportive evidence for this includes histopathological studies showing there is gliosis, necrosis or infarction damaging white matter around areas of CMB-related atherosclerotic or amyloid deposition damage [284, 321]. CMB have also been shown to be

correlated with white matter ultrastructure damage on diffusion tensor imaging (DTI) [313].

We did not find an association between CMB and incident dementia. Ding et al. [288] reported higher dementia risk in their participants with three or more microbleeds. In models adjusted for other SVD markers, two other large population studies, the Framingham [296] and Rotterdam [70] studies reported associations between incident dementia and deep, but not lobar microbleeds. This was explained in two ways; that those with strictly lobar CMB may have lower burden of hypertensive arteriopathy and so may take longer for dementia to develop [296] and, that participants with deep or infratentorial microbleeds often had a higher microbleed count and more mixed microbleed location, potentially representing more severe pathology [70].

The associations of regional CMB with impairments is a significant interest of current research [70, 311, 320, 322]. In a meta-analysis, Wu et al. [287], reported associations of deep and lobar CMB with cognitive impairment, but not infratentorial lesions. There may also be a differential expression of cognitive impairment depending on region, potentially related to the difference in underlying pathology, with CMB in the subcortical and deep regions being associated with atherosclerotic small vessel disease and CMB in lobar regions more likely associated with CAA [68, 69]. Different geographical populations also have a different distribution patterns of CMB, with mainly lobar CMB in Western populations and mainly deep or infratentorial in Asian populations [323]. An additional mechanism for CMB-associated cognitive impairment is the contribution of amyloid pathology through CAA, a very common pathological finding in AD (Martinez-Ramirez et al. 2014). The fact that visuospatial decline was seen even after adjustment for WMH and other markers of CVD disease and this relationship was sustained in those with strictly lobar CMB suggests that AD pathology may be a contributory factor. With significant AD pathology, we may have expected to see an association with memory impairment however, which did not occur.

The main limitation of our study was its size and although sufficiently powered for the main analysis, it was underpowered to look at the relative contribution of those with multiple CMB, with some recent studies suggesting this group plays a disproportionate role in cognitive impairment and dementia [70, 288, 315]. It was also underpowered to detect differences in those with a strictly deep distribution of microbleeds, a relatively rare finding in our cohort, with only four participants in this group. Another limitation was the attrition of the sample, with those not followed up being more cognitively impaired at baseline and their absence from the longitudinal data being a probable source of bias. Survival bias is a common issue in longitudinal studies, with participants who drop out of observational cohort studies having an increased likelihood of progression to dementia [324], suggesting that our results may be an underestimate of the true effect. Eleven percent attrition over four years is reasonable and the use of mixed modelling techniques best accounts for missing data. Finally, we were not able to include an AD biomarker, such as amyloid PET. This could help delineate the relative contributions of CVD and AD pathology to cognitive impairment [9], via the presence of CMB.

Strengths of this study were its ability to control for a number of moderating and confounding factors. Full neuropsychological evaluation allowed detailed assessment of different cognitive domains and the use of multiple tests within a cognitive domain gave a more robust assessment. The use of SWI also allows a much greater detection of potential CMB less sensitive sequences such as gradient recalled echo (GRE) [325].

Further longitudinal studies are required to elicit the nuanced contribution of CMB to cognitive impairment and dementia, particularly with respect to individual cognitive domains. Future studies should be large enough to stratify the number of CMB into meaningful categories and the use of multicenter collaborations is recommended. This should allow meta-analysis [326] and account for the role of geographical difference and variations in neuropsychological tests in CMB associated cognitive impairment. CMB are an important independent predictor of specific cognitive impairments in an ageing population cohort and should be included when considering the burden of cerebrovascular disease.

Informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of the investigation. Informed consent was obtained from all patients for being included in the study.

Supplemental tables

Supplemental Table 2.6. Longitudinal relationship of *strictly lobar* cerebral microbleeds (CMB) with changes in cognition; expressed as estimated marginal means, adjusted for age, sex and education (Model 1; N=302[a], number with CMB=60).

Cognitive domain		Estimated marginal z-score mean [a]			Interaction contrast representing difference between CMB groups in change from W2 to W4		
		Wave 2	Wave 3	Wave 4	Value (CI)	t	p-value
Global Cognition	CMB absent	0.07	-0.07	-0.34			
	CMB present	-0.07	-0.24	-0.65	0.17 (-0.02 - 0.37)	1.73	0.085
	CMB absent	0.07	-0.07	-0.34			
	Multiple CMB (≥ 2)	-0.19	-0.22	-0.70	0.1 (-0.21 - 0.41)	0.64	0.521
Attention and Processing speed	CMB absent	0.03	-0.15	-0.38			
	CMB present	0.02	-0.31	-0.63	0.24 (-0.03 - 0.52)	1.76	0.079
	CMB absent	0.03	-0.15	-0.38			
	Multiple CMB (≥ 2)	-0.20	-0.28	-0.46	-0.15 (-0.57 - 0.27)	-0.69	0.491
Executive function	CMB absent	0.03	-0.12	-0.31			
	CMB present	-0.22	-0.39	-0.44	-0.12 (-0.35 - 0.11)	-1.03	0.304
	CMB absent	0.03	-0.12	-0.31			

	Multiple CMB (≥ 2)	-0.39	-0.43	-0.45	-0.28 (-0.63 - 0.07)	-1.59	0.113
Language	**CMB absent**	-0.07	-0.09	-0.26			
	CMB present	-0.05	-0.21	-0.36	0.13 (-0.1 - 0.36)	1.12	0.265
	CMB absent	-0.07	-0.09	-0.26			
	Multiple CMB (≥ 2)	0.00	-0.23	-0.46	0.27 (-0.09 - 0.63)	1.47	0.143
Visuospatial	**CMB absent**	0.24	0.14	-0.10			
	CMB present	0.03	-0.03	-0.59	0.28 (0.02 - 0.53)	2.11	0.036
	CMB absent	0.24	0.14	-0.10			
	Multiple CMB (≥ 2)	-0.05	-0.03	-0.78	0.39 (-0.02 - 0.8)	1.89	0.059
Memory	**CMB absent**	0.02	-0.02	-0.21			
	CMB present	-0.05	-0.12	-0.30	0.03 (-0.21 - 0.26)	0.22	0.828
	CMB absent	0.02	-0.02	-0.21			
	Multiple CMB (≥ 2)	-0.09	-0.14	-0.21	-0.11 (-0.47 - 0.25)	-0.60	0.552

[a] - at wave 2.

Supplemental Table 2.7. Longitudinal relationship of cerebral microbleeds (CMB) with changes in cognition; expressed as estimated marginal means, adjusted for age, sex and education (N=302[a], number with CMB=60).

	Executive function						Visuospatial ability					
	Model 2			Model 3			Model 2			Model 3		
	Contrast estimate for difference between groups in change from W2 to W4 (CI)	t	p-value	Contrast estimate for difference between groups in change from W2 to W4 (CI)	t	p-value	Contrast estimate for difference between groups in change from W2 to W4 (CI)	t	p-value	Contrast estimate for difference between groups in change from W2 to W4 (CI)	t	p-value
Whole Brain												
CMB present	-0.18 (-0.39 - 0.02)	-1.79	0.074	-0.17 (-0.37 - 0.04)	-1.58	0.114	0.28 (0.05 - 0.50)	2.43	0.016	0.28 (0.05 - 0.50)	2.41	0.016
Multiple CMB	-0.27 (-0.53 – 0.00)	-1.99	0.047	-0.26 (-0.53 – 0.00)	-1.96	0.051	0.29 (-0.01 - 0.59)	1.90	0.059	0.29 (-0.01 - 0.59)	1.89	0.060
Strictly Lobar Distribution												
CMB present	-0.16 (-0.4 - 0.07)	-1.37	0.172	-0.14 (-0.38 - 0.10)	-1.14	0.255	0.3 (0.04 - 0.56)	2.27	0.024	0.3 (0.04 - 0.56)	2.25	0.025
Multiple CMB	-0.28 (-0.64 - 0.07)	-1.58	0.115	-0.28 (-0.64 - 0.07)	-1.57	0.117	0.4 (0.00 - 0.81)	1.96	0.050	0.4 (0.00 - 0.81)	1.95	0.051

Model 2 - adjusted for age, sex, educational level and cardiovascular risk score, APOE-4 status

Model 3 - adjusted for age, sex, educational level and cardiovascular risk score, APOE-4 status, WMH and lacunes.

[a] - at wave 2.

2.3 Summary of main findings

On cross-sectional analysis, I found there was a significant association between the presence of CMB and poorer executive function, even when adjusted for demographic and vascular risk factors. However, this finding was not sustained longitudinally. In contrast, it was those participants with CMB at baseline that had a greater decline in visuospatial function. CMB was not associated with incident dementia. Executive function is one of the most commonly reported domains to be impacted by SVD, supporting the argument that CMB may be a proxy of generalised vascular disease and its subsequent sequalae. I also found regional differences in that individuals with a strictly lobar distribution of CMB did not have impaired executive function.

Most of the results remained significant after adjusting for the presence of WMH. This suggests an independent effect for CMB on cognition, which supports the idea of an index; that several measures together may explain more of the variability seen in cognition. The study suggested that the presence of multiple microbleeds, rather than any (ie, >0) is more strongly associated with cognitive impairment, possibly due to the poor reliability of the rating of just one CMB in the brain. This is significant when considering the constitution of an index.

Chapter 3: Development and validation of a rating scale for dilated perivascular spaces on MRI

3.1 Overview

After considering the contribution of CMB, in Chapter 3, I moved on to examine the impact of another common SVD marker, dilated PVS. After CMB, these were the most common variables identified in the systematic review and update – in 5 of the 12 indices. PVS are fluid spaces that surround small blood vessels as they travel through the brain parenchyma and are commonly microscopic[38]. When dilated, they are visible on conventional imaging and are very common, particularly in the elderly, where their presence has been found in up to 100% of community dwelling older participants[327]. Once thought to be a normal variant, there is increasing evidence of their association with SVD and neurodegenerative pathology[328].

There have been many rating scales developed to quantify the severity of PVS pathology[329]. Most have been developed for T2 weighted images, where PVS present as hyperintense signals. I was unable to use any of these scales reliably – with poor interrater and intrarater reliability found. I therefore developed my own scale, with better psychometric properties, to allow the assessment of PVS pathology.

I developed a simple rating scale, based on the absolute count of PVS in two separate axial MRI slices. These slices were in the basal ganglia and centrum semiovale respectively, two regions where PVS are commonly found. The specific axial slices were chosen as they had the highest average number of PVS within that region.

3.2 Publication: Paradise MB, Beaudoin MS, Dawes L, et al. Development and validation of a rating scale for perivascular spaces on 3T MRI. J Neurol Sci 2020;409:116621.

Development and validation of a rating scale for perivascular spaces on 3T MRI

Abstract

Background and Purpose:

To develop and validate a novel perivascular space rating scale, based on single axial slices in the basal ganglia and the centrum semiovale on T1-weighted and FLAIR images obtained on a 3T MRI scanner.

Methods:

414 community dwelling older adults aged 70-90 were assessed. The number of perivascular spaces in the slices 2 mm (basal ganglia) and 37 mm (centrum semiovale) above the anterior commissure were counted. The construct validity of the scale was tested by examining associations with age, sex, vascular risk factors and neuroimaging markers of small vessel disease; white matter hyperintensities, lacunes and cerebral microbleeds. Associations with cross sectional global and domain specific cognition were also examined.

Results:

The rating scale had excellent inter-rater reliability (intraclass correlation coefficient in basal ganglia 0.82 and centrum semiovale 0.96), good intra-rater reliability (ICC in basal ganglia 0.72 and centrum semiovale 0.87) and reasonable concurrent validity with an existing perivascular spaces scale (Spearman rho = 0.49, p <0.00l). There was a median of four basal ganglia and zero centrum semiovale perivascular spaces. Basal ganglia perivascular spaces were more common in men and associated with the other neuroimaging markers. Perivascular spaces in either location were not independently associated with global or domain specific cognitive impairment.

Conclusion:

The new rating scale is easy to use, quick, has good psychometric properties and performs better than existing scales in a community dwelling older cohort. Further

studies are needed to validate the scale in more diverse cohorts with greater cerebrovascular burden.

Abbreviations:

PVS = perivascular spaces, SVD = small vessel disease, CVD = cerebrovascular disease, BG = basal ganglia; CSO = centrum semiovale, SD = standard deviation, BMI = body mass index; WMH = white matter hyperintensity, CMB = cerebral microbleeds.

Introduction

Perivascular spaces (PVS), also known as Virchow-Robin spaces[330, 331] are fluid spaces that surround small blood vessels as they travel through the brain parenchyma. The perivascular space is responsible for drainage of interstitial fluid and may have a role in normal neural homeostasis and removal of toxins and immunity through the glymphatic system[332, 333]. Once thought to be a normal variant, there is increasing evidence for the association of MRI-visible PVS with cerebral small vessel disease (SVD)[59, 62, 334] and neurodegenerative[61, 63] pathology. There is an inconsistent association with cognitive impairment[327, 328]. Hypothesized etiologies for dilated PVS include hypertension, obstruction, inflammation and atrophy[64].

There are several published PVS rating scales (Table 3.1), which generally assign a grade of severity to the number of PVS in specific locations and/or anatomical slices. Both T1 and T2-weighted images are needed to distinguish PVS from lacunes, the former characteristically demonstrating a hyperintense rim on T2-weighted or FLAIR imaging. Most of the scales are based on T2-weighted images as PVS have CSF signal intensity and are hyperintense and easier to visualize on these sequences[187]. However, if a clinician or researcher does not have access to T2-weighted scans but only T1-weighted and FLAIR sequences, a common scenario, the applicability of existing scales can be limited. Only the Zhu et al.[59] scale used T1-weighted images as the primary rating sequence, developed with a 1.5T scanner and this proved to have poor inter-rater reliability when we used it with images derived from a 3T scanner. We obtained weighted kappas of 0.51 for agreement between ratings of basal ganglia PVS severity and 0.34 for agreement between ratings of centrum semiovale severity. This may be due to high field strength machines (3T) and high-definition sequences increasing the visibility of small PVS, which could lead to decreased observer agreement[187]. Whilst there is active interest in developing automated methods for detection and measurement of PVS[63, 335, 336], these techniques are not sufficiently mature or reliable for routine clinical use.

Table 3.1. Comparison of visually rated PVS scales (excluding automated or semi-automated quantitative methods)

Study	Subjects	MRI sequence and slice thickness (ST)	Rating methods
Heier 1989[337]	816 outpatients undergoing MRI of all ages.	1.5T three spin echo sequences (1) axial ST=5 mm , 5mm gap; (2) axial multiecho images ST= 5 mm, 2.5-mm gap; (3) sagittal ST= 5mm, 5-mm gap.	Severity grade by size (diameter). Mild (< 2 mm), moderate (2-3 mm) and severe (> 3 mm).
Adachi 2000[338]	171 patients admitted with acute cerebral infarcts	1.5T T2, T1 and proton density all axial with ST = 6mm, no gap.	4-level severity score of number of BG PVS- Grade 0, no PVS; grade 1, 1-5 PVS; grade 2, 6-10 PVS; grade 3, >10 PVS
Di Costanza 2001[339]	41 adults with myotonic dystrophy – mean age 39	0.5T sagittal T1, axial proton density and T2 all with ST=5 mm, 2mm gap.	Lenticulostriate or convexity VRS. : Number multiplied by size category, where size 1 = <2mm, 2 = 2-3mm and 3 = >3mm
Maclullich 2004[60]	97 healthy men (65–70 years)	1.9 T T2 with ST = 5 mm, 1mm gap.	5-level severity score for hippocampus, BG and CSO (0=no PVS, 1=<10 PVS, 2=10–20 PVS, 3=21–40 PVS, and 4=>40 E=PVS. Count on most affected hemisphere.
Pantankar 2005[340]	35 ppts with Alzheimer's, 24 with vascular dementia, 16 with fronto-temporal	1.5T axial FLAIR. ST=3 mm with no gap. 3D T1 FAST field-echo.	Different severity scores in total BG, CSO and subinsular regions. Binary rating in midbrain.

	dementia and 35 controls.		
Groeschel 2006[58]	125 young health subjects (0.5-30 years)	1.5T T1-weighted 3D high resolution sequence with axial ST = 1 mm.	All PVS counted and location noted (supratentorial white matter and basal ganglia). PVS then classified as 'dilated' or 'not dilated' on basis of shape.
Rouhl 2008[334]	165 first ever lacunar stroke patients	1.5 T axial T2 and FLAIR with ST = 5 mm, 0.5 mm gap.	3-level severity score for total number of PVS in both hemispheres in BG and CSO region (Low - lower than 20 PVS; moderate - between 20 and 50 PVS and high - higher than 50 PVS.
Doubal 2009[62]	350 patients; 129 lacunar, 124 cortical stroke, and 97 age-matched control subjects	1.5-T axial DWI, T2-weighted, FLAIR, and sagittal T1.	5-level severity score for most severe slice (counted on one side) in BG and CSO (0, <10, 11-20, 21-40, >40).
Zhu 2011[59]	1826 ppts from general elderly population study	1.5T 3D high-resolution T1 with ST=1 mm. T2- and proton attenuation images with ST=3.5 mm, 0.5 mm gap.	4-level severity score based on single slice in BG and for WM, a combination of total region and most severe slice. Large (>3mm) PVS were counted separately.

Adams 2013[188]	125 ppts from 2 population-based cohort studies, mean ages 69 and 64.	1.5T axial 3D T1 ST=1.6 mm, T2* ST=1.6 mm, and FLAIR with ST=2.5 mm.	Count on single pre-defined slice. CSO – slice 1 cm above the lateral ventricles. BG - slice showing the anterior commissure. Maximum count of 20. Large (>3mm) PVS counted separately. In hippocampus and midbrain, all unique PVS were counted.
Potter 2015[187]	60 ppts from two studies: ageing population and stroke.	T2. With T1 and FLAIR also available.	5- level severity score in BG & CSO (0; 1-10; 11-20; 21-40; >40 PVS). Binary in midbrain. Most severe slice, counting on one side.
Ding 2017[341]	2612 participants aged 65–97 years from prospective population based study.	1.5T 3D T1 spoiled-gradient echo sequence ST= 1.5 mm. Proton density /T2 FSE sequence, FLAIR, and T2* all with ST= 3.0 mm.	Number of large (>3mm diameter) PVS in BG complex and WM along paths of the perforating medullary arteries.

We therefore aimed to develop a suitable PVS severity rating scale with good psychometric properties, which would be suitable for 3T, high-definition imaging using T1 as the primary imaging sequence of interest. Further, we wished to establish the validity of the rating scale by examining the association with vascular risk factors, neuroimaging markers and a comparison with an existing PVS scale.

Methods

Subjects:

Participants were drawn from the population based longitudinal Sydney Memory and Ageing study[151], an ongoing study which began in 2005 and focuses on cognitive decline in the community-dwelling elderly. Subjects were aged 70-90 years, living in the community and able to complete their assessments in English. Exclusion criteria were major psychiatric or central nervous system disorder. There have been six waves of this study, two years apart. At each wave, participants underwent an MRI scan, comprehensive neuropsychological assessment, medical examination, blood collection, and *APOE* genotyping (wave 1). For this study, we used data from wave 2, when a more comprehensive MRI protocol was introduced. Written consent was obtained from all participants. The methodological details and ethics approval of the study have been previously published[151].

Neuropsychological assessment, administered by trained research psychologists, consisted of a battery of tests grouped into cognitive domains: attention and processing speed (Digit Symbol-Coding [297], and Trail Making Test (TMT) A [298]), memory (Logical Memory [299], Rey Auditory Verbal Learning Test (RAVLT), [298] and Benton Visual Retention Test [300]), language (Boston Naming Test – 30 items [301], Semantic Fluency (Animals) [298]), visuospatial (Block Design [302]), and executive function (Controlled Oral Word Association Test (FAS) [298] and Trail Making Test (TMT) B [298]). Raw test scores were transformed to z-scores using the baseline mean and SD values of a healthy reference subsample (n=723 MAS participants). Domain scores were calculated by averaging the z-scores of component tests (except for visuo-spatial which was represented by a single test) and a global cognition score was calculated by averaging

the domain scores. These composite scores were standardized so that the healthy reference group had means and SDs of 0 and 1 respectively.

Radiological examination:

MRI was performed on a Philips 3T Achieva Quasar Dual scanner (Philips Medical Systems, Best, Netherlands). A 3D T1-weighted sequence (1×1×1 mm3, TR/TE=6.39/2.9 ms), a T2-weighted fluid attenuation inversion recovery (FLAIR) sequence (TR/TE/TI=10000/110/2800 ms; thickness 3.5 mm; 0.898×0.898 mm2) and a SWI sequence (parameters previously described[71]) were performed. Total white matter hyperintensity (WMH) volume was assessed with automated methods using FLAIR and T1-weighted images, and adjusted for total intra-cranial volume (ICV)[40]. Total ICV was assessed using Freesurfer (https://surfer.nmr.mgh.harvard.edu/fswiki/eTIV)[342]. All images were co-registered using Statistical Parametric Mapping version 5 (SPM5). All images were analyzed using MRIcron version 15 (www.nitrc.org/projects/mricron/). When a measuring tool was required, itk-SNAP version 3.6.0 (www.itksnap.org) was used[343]. Lacunes and cerebral microbleeds (CMB) were defined using STRIVE criteria[38].

Development of rating scale:

PVS were defined according to STRIVE[38] criteria, i.e. they have CSF signal intensity and follow the course of penetrating vessels, appearing linear when viewed parallel to the course of a penetrating vessels or round or ovoid when imaged perpendicular to the vessel. They were distinguished from lacunes by the lack of a hyperintense rim on FLAIR sequences. In contrast to PVS, lacunes are usually greater than 3mm in diameter but we included PVS of any diameter if they fulfilled other diagnostic criteria. WMH may present as hypointense lesions on T1-weighted images and comparing these to the FLAIR images helped to exclude these potential mimics. A conservative approach was taken and ambiguous areas were not counted as PVS, with several guide images used by both raters (examples in Figure 1). Where there were 20 or greater PVS in a region, including état criblé, this was recorded as ≥20, rather than requiring the total number to be counted.

Figure 3.1. A. Representative slices in BG and CSO. B. An example of background granularity of CSO region. No hypointense areas were labelled as PVS. C. An example of ambiguous BG PVS. The three identified PVS circled. D. An example of ambiguous BG PVS. The three identified PVS circled. E. An example of severe CSO PVS pathology, with 16 PVS circled.
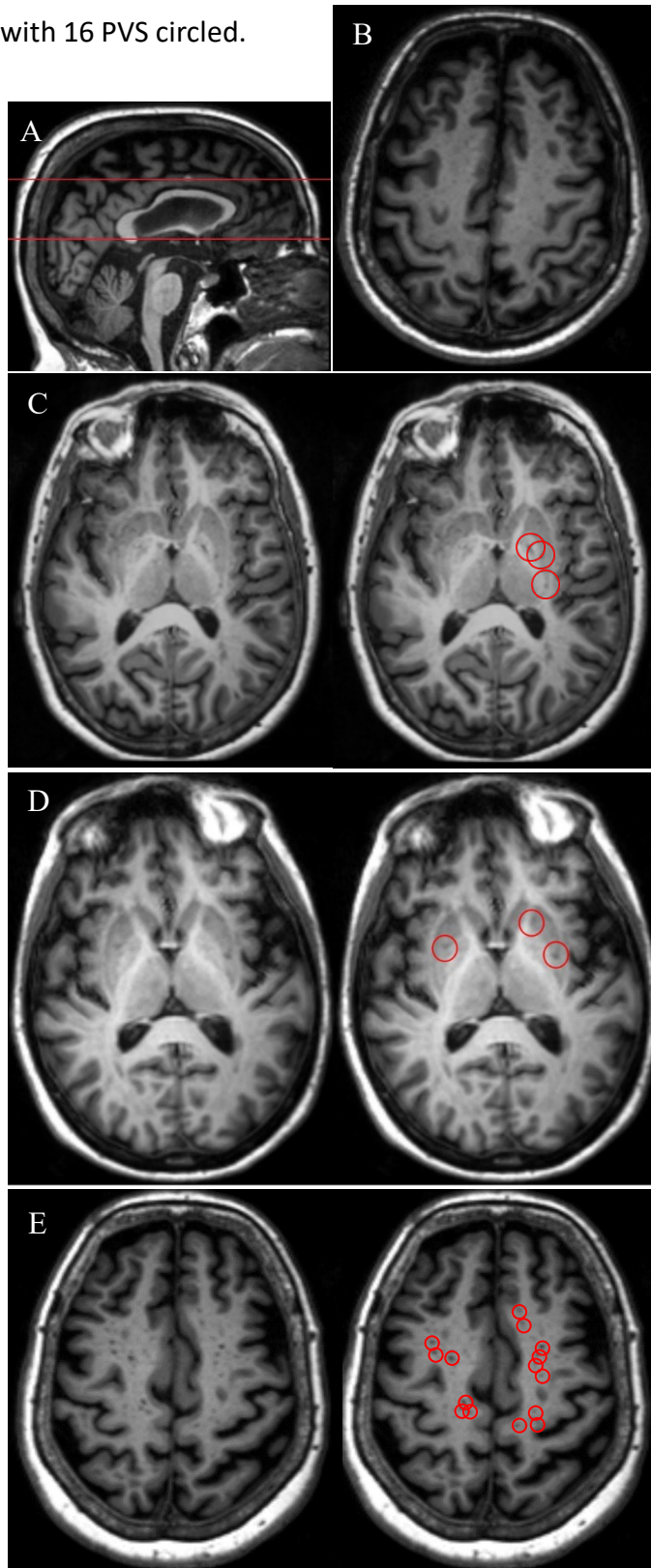
Based on existing scales, we chose to rate PVS in two areas where PVS are commonly found – the basal ganglia (BG) and the centrum semiovale (CSO)[59, 62, 187, 188, 334]. On a sub-sample of 125 participants, for each of these regions, we counted the number of PVS in the axial slice with the greatest severity (i.e., most numerous PVS). This method produced poor inter-rater reliability (intraclass correlation coefficient of 0.26 for BG PVS and 0.36 for CSO PVS). We then chose to use pre-defined slices based on these results, generating the mean distance (slice number in 1mm increments) from the anterior commissure to the slice with the greatest pathology in the BG and CSO respectively. This method produced a BG slice of 2 mm about the anterior commissure (AC) and a CSO slice 37 mm above the AC (Figure 1). The CSO slice was on average 6mm superior to the superior border of the lateral ventricle (SD 3.4mm). In a small number of cases, (16 participants, 4%) this pre-defined CSO slice was at a level where the lateral ventricles were still visible. In these cases, the slice immediately superior to the lateral ventricles was selected. A variety of modifications to the scale were then considered. Midbrain PVS (total number anywhere in the midbrain) were not significantly associated with vascular risk factors or imaging markers and were chosen not to be included in the final scale. Assessing only larger (>3mm) lesions was considered but very few PVS >3mm were identified from the two representative slices in our cohort (five in the BG, two in the CSO). With such limited numbers, no further meaningful analysis could be performed and the use of only larger lesions, or separating lesions by size was abandoned.

Statistical methods:

Two raters, a psychogeriatrician (MP) and a neuroradiologist with over 10 years' experience (LD), independently viewed a random selection of 20 anonymized scans and counted the number of PVS in the BG and CSO slices. This was used to determine the inter-class correlation coefficient for inter-rater reliability. To calculate intra-rater reliability, MP re-rated a random selection of 20 scans three months apart. With a relatively healthy older cohort, to ensure reliability of the scale over a wide range of CVD burden, the reliability analysis was repeated with those individuals (n=14) who would be later diagnosed with vascular dementia, using their most proximate scan to the diagnosis.

Validity assessment: On a subsample of 125 participants selected at random, we assessed concurrent validity by measuring correlation between the new rating scale and the Zhu et al[59]. categorical rating scale with a Spearman correlation coefficient. Construct validity, that the new scale was a measure of PVS burden in the brain and a marker of SVD, was established by examining the associations with demographics, medical characteristics and known neuroimaging markers of SVD.

First, the numbers of PVS in the BG and CSO selected slices were counted. To examine the associations between PVS and demographic and medical characteristics, the distributions of PVS frequency were examined and were found to be non-normal. Based on visual inspection of the scatter plots of PVS number vs. the variables of interest, we preferred to analyze PVS as a continuous variable. There were no obvious cut-points and not using categorical binning avoided loss of information.

Descriptive statistics for sample characteristics were presented using means and SDs for continuous variables and frequencies and percentages for categorical variables. Spearman correlation coefficients were produced for relationships between BG and CSO PVS number and continuous variables. Linear regression analysis was performed for results flagged as significantly correlated with normally distributed dependent variables and then repeated, controlling for age, sex and total ICV. We decided to adjust for these covariates a-priori as other studies have found association between them and PVS. WMH volume was log transformed to more closely approximate the normal distribution. For relationships of dichotomous variables with the number of PVS, the median number of PVS in each category was presented and logistic regression analyses performed to determine if there were any significant differences between groups. The analysis was then repeated controlling for the same covariates as above.

Distributions of the dependent variables of cognition were inspected for normality and to minimize the influence of extreme values on statistical outcomes, scores were winsorized where necessary so that upper and lower values were reduced to three standard deviations above or below the mean. Spearman correlation coefficients were then used to examine associations between the number of BG and CSO PVS and cognition and regression analyses performed, repeated controlling for age, sex, education and total ICV. An alternative analysis was also performed, creating

dichotomous variables for those with the top quartile of BG and CSO severity (vs. lower three quartiles) and t-tests used to examine the association of these two variables with cognition.

Results were Bonferroni corrected for multiple-testing, with α set at 0.017, considering demographics, vascular risk and neuroimaging markers of CVD as three families of variables. All analyses were performed using the SPSS statistical package (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp).

Results

Sample characteristics are presented in Table 3.2 (mean age 79.6, [SD 4.6]; 196 [47%] men). Of the 414 participants, 379 (92%) had at least one PVS; 359 (87%) had at least one BG PVS with a median number of 4 (IQR 2-6); and 184 participants (44%) had at least one CSO PVS with a median number of 0 (IQR 0-1). The frequency histogram is displayed in Figure 3.2.

Table 3.2. Characteristics of the study population (N=414).

	N (%) or Mean ± SD
Total	414
Age, years	79.6 ± 4.6
Men	196 (47)
Education, years	11.9 ± 3.7
Mini-mental state examination	28.2 ± 1.7
BMI[a]	26.9 ± 4.0
Smoker (in last month)[b]	19 (5)
Hypertension[c]	329 (80)
Diabetes[d]	81 (20)
Cardiovascular Disease Risk Factor Score[e]	3.8 ± 3.3
APOE-ε4 carrier[f]	95 (23)
Total WMH volume mm^3	15,319 ± 14,296
Presence of lacunes	35 (9)
Presence of CMB[g]	80 (22)

Data are missing for [a] 10 [b] 76 [c] 2 [d] 1 [e] 15 [f] 2 [g] 54 participants respectively.

Figure 3.2. Frequency distribution of BG and CSO PVS.
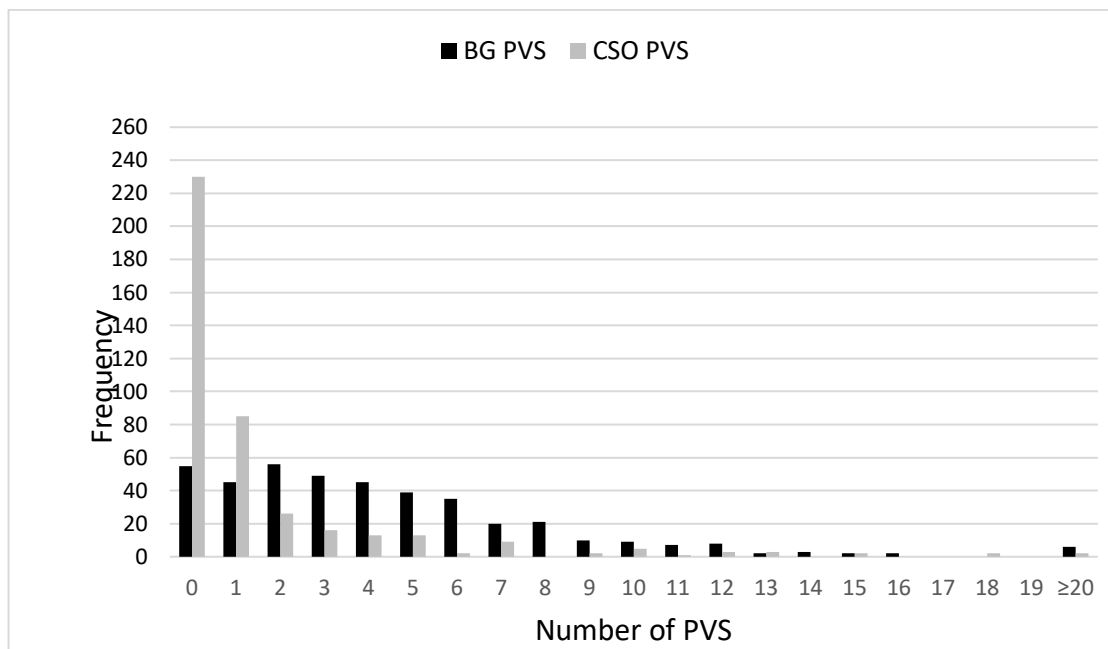
Number of PVS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	≥20
BG PVS frequency	55	45	56	49	45	39	35	20	21	10	9	7	8	2	3	2	2	0	0	0	6
CSO PVS frequency	230	85	26	16	13	13	2	9	0	2	5	1	3	3	0	2	0	0	2	0	2

Reliability and validity: There was excellent[344] inter-rater reliability with an intraclass correlation coefficient of 0.82 for the BG PVS and 0.96 for the CSO PVS ratings. There was good[344] intra-rater reliability with an intraclass correlation coefficient of 0.72 for the BG PVS and excellent reliability at 0.87 for the CSO PVS intra-reliability rating[344]. For the sample of individuals with more severe CVD, the inter-rater reliability intraclass correlation coefficient was slightly lower but acceptable at 0.65 for the BG PVS and 0.87 for the CSO PVS ratings. The intra-rater reliability was excellent [344] with an intraclass correlation coefficient of 0.91 for the BG PVS and excellent reliability' at 0.83 for the CSO PVS intra-reliability rating[344]. The use of the vascular diagnosis as a proxy for more severe CVD appeared valid, with these individuals having on average 36% greater WMH and larger numbers of BG-PVS (7) and CSO PVS (6)

Concurrent validity, as assessed by comparison with the Zhu et al.[59] scale, revealed a significant correlation between the number of BG PVS and the Zhu rating of BG PVS severity (ρ = 0.49, p <0.00l). There was also a significant correlation between CSO PVS and the Zhu rating of CSO PVS severity (ρ = 0.55, p <0.00l). There were no significant differences between the characteristics of the subsample of 125 used for the concurrent validity assessment and the main group (N = 414). The predetermined slices generated by our method had a standard deviation of 3.6mm (BG) and 7.4mm (CSO) of variability respectively around the slice with the most severe pathology selected by the Zhu et al.[59] scale. The scale was easy-to-use, with identification of the anterior commissure, selection of the two representative slices and examining the T1 weighted image side-by-side to the FLAIR taking an average time of three minutes per participant. This compared favorably with the Zhu et al.[59] scale, with an average time of five minutes per participant as the whole of the white matter needed to be examined.

As seen in Table 3.3, BG PVS were more common in men (B=0.07 OR 1.07 (95% CI 1.07 to 1.13), Wald 6.70, p=0.010), associated with greater WMH volume (log transformed; B=0.02 (95% CI 0.01 to 0.03), β=0.25, t = 5.14, p <0.00l), more numerous in the presence of cerebral microbleeds (B=0.13 OR 1.14 (95% CI 1.07 to 1.21), Wald 16.24, p <0.00l) and showed a trend to be more numerous in the presence of lacunes (B=0.09 OR 1.09 (95% CI 1.01 to 1.18), Wald 5.36, p=0.02 - did not achieve significance after

Bonferroni correction). There were no other associations between PVS and demographic, medical and vascular risk factors and neuroimaging markers. For significant and trend associations, adjusting for age, sex and ICV did not change results, with the exception of the relationship with BG PVS and lacunes. Here, the relationship weakened, due to the effect of male sex, which was strongly associated with the presence of lacunes (B=0.08 OR 1.08 (95% CI 0.998 to 1.17), Wald 3.63, p=0.06).

Table 3.3. Associations between PVS and demographic, vascular risk factor and imaging markers (N=414).

	BG PVS (n=359)		CSO PVS (n=184)	
	Correlation coefficient (Spearman's rho)	Median number of PVS	Correlation coefficient (Spearman's rho)	Median number of PVS
Demographics				
Age, years	0.08	-	0.01	-
Sex - Male	-	4**	-	0
- Female	-	3	-	0
Education, years	0.01	-	0.03	-
Mini-mental state examination, mean	-0.01	-	0.05	-
Total intracranial volume	0.09		0.01	
Vascular risk				
BMI	-0.08	-	-0.02	-
Cardiovascular Disease Risk Factor Score	0.08	-	0.01	-
Smoker (in last month)	-	3	-	1
- Non-smoker	-	4	-	0

Hypertension, - presence	-	4	-	0
- Absence	-	4	-	0
Diabetes - presence	-	4	-	0
- Absence	-	3	-	0
APOE-ε4 carrier, presence	-	3	-	0
- non ε4 carrier	-	4	-	0

Neuroimaging markers of CVD

Total WMH volume mm^3	0.26***	-	0.06	-
Lacunes - presence	-	6*	-	0
- absence	-	3	-	1
CMB - presence	-	5***	-	0
- absence	-	3	-	0

*p<0.05; **p<0.01; ***p<0.001. Bonferroni corrected: critical p-value = 0.017.

The only significant association between BG or CSO PVS and either global cognition or individual cognitive domains (Table 3.4) was between the number of CSO PVS and memory (Spearman's rho 0.10, p = 0.04). When adjusted for age, sex, education and ICV in a regression analysis, this was no longer significant (B=0.02 95% CI -0.10 to 0.05, t=1.35, p=0.18). When categorized as dichotomous variables, participants with the most severe BG PVS (top quartile; number ≥ 6) or the most severe CSO PVS (top quartile; number ≥ 2) were not any more likely to have impairments in global cognition or any cognitive domain compared to those with less severe or absent PVS. This includes a lack of association between severe CSO PVS and memory; difference in z-score -0.18 (95% CI, -0.43 to 0.08), t=-1.39, p=0.17).

Table 3.4. Associations between PVS and cognition (N=414).

	BG PVS (n=359)	CSO PVS (n=184)
	Correlation coefficient (Spearman's rho)	
Global cognition	-0.03	0.06
Attention and Processing Speed	-0.02	0.03
Executive function	-0.01	0.01
Language	0.02	0.04
Visuospatial function	-0.06	0.03
Memory	-0.01	0.10*

*$p < 0.05$

Discussion

We have developed a new rating scale for PVS, based on high-definition/field strength imaging using T1 and T2-FLAIR images. The total PVS counts in single predetermined slices in the BG and CSO formed the basis of the measurement. Our scale differs from other rating scales (Table 3.1) as the only scale where the pre-defined slice was selected through an empirical process. Using a pre-defined slice improved inter-rater reliability, and with our cohort the scale performed better than existing scales in terms of reliability and speed of use. Including all PVS regardless of size also saved time as there was no need to measure individual PVS and with these two features the scale was quick, taking 3 minutes per individual (approximately half that of the Zhu et al.[59] scale). We found support for the predefined single slice approach with reasonable concurrent validity with the Zhu et al.[59] rating scale, which assessed PVS in 4 grades counting PVS in the whole WM if total PVS count was <10, else if ≥10 in the slice containing the greatest number of PVS. Others have also found a high correlation (0.79) between a single slice approach and the total number in PVS in a given region[64].

We found that BG PVS are common in an older population (mean age 80 years, range 72-92) with a median of four PVS, and that CSO PVS are less common, with under one-half of participants having one or more lesions identified. BG PVS were more common in men and strongly associated with the neuroimaging markers of WMH volume and the presence of cerebral microbleeds, but had a non-significant association with presence of lacunes after Bonferroni correction. There were no associations between either BG or CSO PVS and cognition.

There are very divergent results reported for the association of PVS with vascular risk factors and demographic factors[62, 334, 337, 341, 345-348]. Some of this inconsistency is due to the heterogeneity of definitions and rating scales used to quantify PVS. This is further complicated by semantic uncertainty, with the terms 'dilated', 'large', 'prominent' and 'expanded' used to describe the phenomenon and no widespread agreement as to the thresholds for determining a dilated PVS. Some authors considered any visible PVS as being dilated, with others requiring additional criteria, such as a diameter over 1 mm[188] or the morphology of lesions[58]. The STRIVE[38] standardization criteria chose the

term "perivascular space", noting the association between the size of PVS and clinical consequence is not well understood[38]. They described that PVS were generally less than 3 mm in diameter, avoided describing the PVS as a lesion and recommended that terms like 'dilated' and 'enlarged' be avoided as the visibility of PVS depends not only on their actual size but on MRI field strength and MRI sequence characteristics[349]. Notwithstanding, a recent meta-analysis[328] did find a strong association between PVS and age. Our lack of association may be due to our relatively health cohort, which has less severe vascular pathology than in some of the other cohorts (i.e., those post-stroke) and which has a narrower age range than many studies. This makes a statistically significant association less likely to occur. The meta-analysis[328] also found an overall association between hypertension and BG, but not CSO PVS. Even with BG PVS however, similar to our results, over half of the constituent studies did not find an association. The divergent associations of hypertension with PVS may be explained by the challenges of summarizing blood pressure in one variable. The effect of hypertension differs with age and both hyper-and hypotension have sequalae and the location of the PVS within the cerebral vasculature being important, with proximal and distal regions of the cerebral vasculature having different susceptibility to blood pressure gradients[350]. Genetics, chronicity of hypertension and medication effects add to the complexity of the relationship[351].

The association of BG PVS with neuroimaging markers of SVD is consistent with published literature[62, 63, 334, 341, 346, 348, 352] and supports the construct validity of the scale. This suggests BG PVS are a marker of SVD and not just an incidental finding or epiphenomenon of age. In contrast, several studies corroborated our finding that white matter (CSO) PVS were not associated with WMH[334, 345, 348] or lacunes[62]. The weak association between BG PVS and lacunes was mediated by male sex and may also be due to difficulties in differentiating between the two types of marker, which could potentially lead to either type I or II errors.

We found a paucity of CSO PVS relative to BG PVS and a recent meta-analysis of five studies found the inverse – that CSO PVS are generally more numerous than BG PVS. One explanation is that PVS can be difficult to rate and prone to inter-rater variability in the presence of extensive WMH[187]. Reduced CSO PVS numbers would therefore

potentially be counted in both those with minimal and large SVD burdens and confound associations, leading to possible type II errors.

The reported relationship of PVS with cognition is mixed. Several studies have reported association with incident dementia[341] or in dementia sub-groups[63] , but association with cognition cross-sectionally is weaker[345] [60, 160, 353]. Two recent meta-analyses did not find an association[327, 328].

Our differing results for BG and CSO PVS support at least partially distinct aetiologies in these areas. BG-PVS are thought to be a marker for hypertensive arteriopathy, while CSO-PVS might reflect the presence of CAA or a mixed hypertensive / CAA[63, 346, 347, 354] pathophysiology. Recent genetic analysis suggests different heritability patterns for PVS in BG and white matter respectively, further supporting the idea of separate pathophysiology[352].

There were limitations to our study. The lack of associations between CSO PVS and cardiovascular or neuroimaging measures could potentially be due to lack of power, as CSO PVS were less frequent with less variability in their number (i.e., IQR 0-1). Further, although we have suggested that using a single slice is appropriately representative of the regional PVS burden, this may not always be the case due to individual anatomical variation in the location of PVS, particularly for the rarer larger PVS. Given our low numbers of CSO PVS, our pre-selected slice method may under-detect PVS compared to methods which evaluate the total number of PVS in the whole white matter or select a slice with the most severe pathology.

Conclusions

We have demonstrated the validity of new PVS rating scale, derived from T1-weighted and FLAIR images on a 3T scanner, which performed better than existing scales in a cohort of community dwelling older adults. It had good reliability and was quicker to use than existing scales. Further research plans include testing the validity of the scale in different cohorts, including those with greater vascular burden. We hope that the use of a scale will help address key questions about the aetiology of PVS and the utility of this SVD marker in predicting longitudinal cognitive decline.

3.3 Summary of main findings

The newly developed scale counted the number of PVS in slices 2mm and 37mm above the anterior commissure respectively. The scale had excellent interrater reliability (intraclass correlation coefficient in basal ganglia 0.82 and centrum semiovale 0.96), good intrarater reliability (ICC in basal ganglia 0.72 and centrum semiovale 0.87) and reasonable concurrent validity with an existing perivascular spaces scale (Spearman rho = 0.49 p < .001). I found that the new scale performed better than existing scales in a community dwelling older cohort, in terms of speed of use and reliability.

I assessed the contrast validity of the scale by examining associations with age, sex, vascular risk factors and neuroimaging markers of SVD; WMH, lacunes and CMB. Basal ganglia PVS in particular, were associated with the other neuroimaging marker, supporting their aetiology as a marker of hypertensive arteriopathy. To further assess the construct validity of the scale, I examined the association of PVS with cross-sectional cognition and found only a significant association between the number of CSO-PVS and memory (Spearman's rho 0.10, p = 0.04). When adjusted for age, sex, education and intracranial volume in a regression analysis however, this was no longer significant (B = 0.02 95% CI -0.10 to 0.05, t = 1.35, p=0.18).

The weak association between CSO-PVS and memory is intriguing, given CSO-PVS might reflect the presence of CAA or a mixed hypertensive/CAA[329] pathophysiology. In the following chapter, the development of a reliable rating scale allowed more sophisticated assessment of the complex relationship between PVS, cognition and dementia over a follow up period of up to 8 years.

Chapter 4: The association of PVS with longitudinal cognitive decline and incident dementia

4.1 Overview

In Chapter 4, I use the newly developed PVS rating scale (Chapter 3) to examine in detail the complex relationship between PVS, longitudinal decline and incident dementia. I used a sample of 414 older adults from the Sydney Memory and Ageing Study (MAS). Participants were followed up for four years in the assessment of cognitive decline and up to eight years for assessment of incident dementia, using detailed neuropsychological assessment and multidisciplinary consensus diagnosis.

Participants were defined as having severe PVS pathology if they were within the top quartile of PVS number in the BG or CSO slice respectively. For the analysis, four non-exclusive binary PVS predictor variables were created, representing those with severe PVS pathology in i) either region; ii) both regions; and those with iii) severe BG PVS and iv) severe CSO PVS pathology. Linear mixed modelling was used to examine associations of PVS with longitudinal cognitive decline – both domain specific and Global Cognition. Discrete time survival analysis was used to examine associations between PVS and incident dementia over eight years. This approach allowed examination of time interactions at each of the 2-year time periods. Importantly, I adjusted for demographics, vascular risk factors and other SVD markers; WMH volume, presence of multiple CMB and presence of lacunes. This allowed the assessment of the effect of PVS on cognition, independent of other SVD pathology.

With an elderly population and eight years of follow up, there was significant attrition and I used a variety of approaches to minimize this, including sensitivity analysis. Both LMM and the discrete time survival approach better allow analysis of missing dependent variable data for a particular wave, compared with using the more traditional case-wise deletion method.

4.2 Publication: Paradise M, Crawford JD, Lam BCP, et al. The Association of Dilated Perivascular Spaces with Cognitive Decline and Incident Dementia. Neurology 2021:10.1212/WNL.0000000000011537.

The association of dilated perivascular spaces with cognitive decline and incident dementia

Abstract

Objective:

To determine if severe perivascular space (PVS) dilation is associated with longitudinal cognitive decline and incident dementia over four and eight years respectively, we analyzed data from a prospective cohort study.

Methods:

414 community dwelling older adults aged 72-92 were assessed at baseline and biennially for up to eight years, with cognitive assessments, consensus dementia diagnoses and 3T MRI imaging. The numbers of PVS in two representative slices in the basal ganglia (BG) and centrum semiovale (CSO) were counted and severe PVS pathology defined as the top quartile. The effects of severe PVS pathology in i) either region; ii) both regions; and those with iii) severe BG PVS and iv) severe CSO PVS were examined. White matter hyperintensity volume, cerebral microbleed number and lacune number were calculated.

Results:

Participants with severe PVS pathology in both regions or in the CSO alone had greater decline in global cognition over four years, even after adjustment for the presence of other small vessel disease neuroimaging markers. The presence of severe PVS pathology in both regions was an independent predictor of dementia across eight years (OR 2.91, 95%CI 1.43–5.95, p= 0.003). Further, the presence of severe PVS pathology in all groups examined was associated with greater dementia risk at either year four or six.

Conclusions:

Severe PVS pathology is a marker for increased risk of cognitive decline and dementia, independent of other small vessel disease markers. The differential cognitive associations for BG and CSO PVS may represent differences in their underlying pathology.

Introduction

Perivascular spaces (PVS) are fluid spaces that surround small blood vessels within the brain parenchyma. There is increasing evidence for the association of MRI-visible dilated PVS with small vessel disease (SVD)[59, 62, 334] and neurodegenerative[61, 63] pathology.

Few longitudinal studies have examined the association of PVS with incident dementia[61, 341] and a recent review reported ambiguous findings for the association with Alzheimer's disease (AD) or all-cause dementia[355]. There are also mixed findings regarding the association of PVS with cognitive impairment; specifically, our data[329] and two recent meta-analyses failed to find an association with cognitive impairment cross-sectionally[327, 328]. There is stronger evidence for an association between PVS and longitudinal cognitive impairment[61, 341, 356].

PVS are commonly seen in the basal ganglia (BG) and centrum semiovale (CSO). This may represent at least partially different pathophysiology, with BG PVS being associated with more hypertension-related pathology and CSO PVS associated with amyloid pathology and ultimately, higher incidence of AD[63, 357]. These regions may in-turn be associated with impairments in different cognitive domains, resulting from damage to specific cognitive networks and/or due to underlying pathology.

Our primary aim was to determine if total PVS severity (severe PVS pathology in either region) was associated with decline in global cognition and incident dementia. Further, to explore whether severity in both regions, or regional BG or CSO PVS severity was associated with these same outcomes. Our secondary aim was to determine if PVS severity, either totally or specifically in the BG or CSO, was associated with impairments in specific cognitive domains. We aimed to determine if any impairments seen were independent of other neuroimaging markers of SVD, which might suggest an independent pathway for the contribution of PVS to cognitive impairment.

Methods

Subjects:

Participants were part of the Sydney Memory and Ageing study (MAS)[151]. This is a longitudinal, population based study, which started in 2005 and investigates cognitive decline in the elderly. Participants were aged 70-90 years at entry to the MAS, living in the community and able to complete their assessments in English. Exclusion criteria were major psychiatric or central nervous system disorders including schizophrenia. bipolar disorder, motor neurone disease, multiple sclerosis, central nervous system inflammation, developmental disability or any other condition that may have interfered assessment completion[151].

At each wave, 2 years apart, participants had an MRI scan, comprehensive neuropsychological assessment, medical examination and blood collection (including APOE genotyping in wave 1). For this study, we used data from wave 2, when a more comprehensive MRI protocol was introduced, and this was considered baseline for the current study. Our sample of 414 comprised all participants at wave 2 who consented and were eligible to have an MRI scan – see Figure 4.1; Participant Flow Chart. Comprehensive neuropsychological assessment was available for waves 2 to 4 (baseline to year 4 of this PVS study) and dementia diagnosis for waves 2 to 6 (baseline to year 8).

Figure 4.1. Participant flow chart
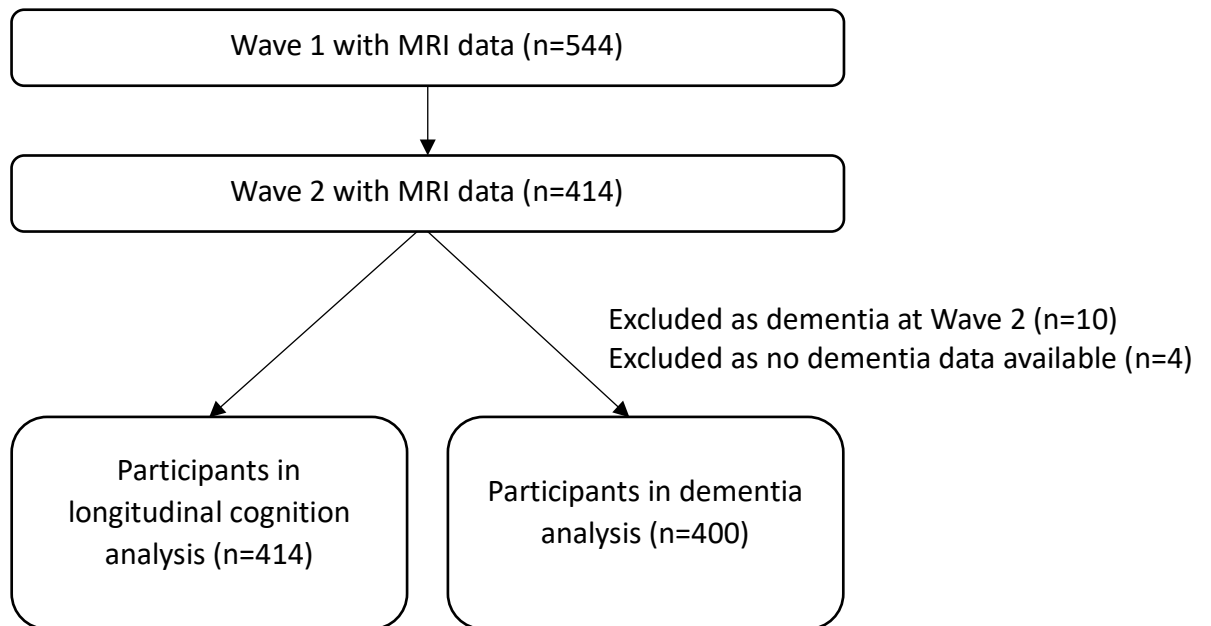
```
┌─────────────────────────────────────────────────┐
│         Wave 1 with MRI data (n=544)             │
└─────────────────────────────────────────────────┘
                        │
                        ▼
┌─────────────────────────────────────────────────┐
│         Wave 2 with MRI data (n=414)             │
└─────────────────────────────────────────────────┘
            /                       \
                    Excluded as dementia at Wave 2 (n=10)
                    Excluded as no dementia data available (n=4)

┌──────────────────────┐      ┌──────────────────────┐
│    Participants in    │      │ Participants in dementia │
│ longitudinal cognition│      │   analysis (n=400)    │
│   analysis (n=414)    │      │                       │
└──────────────────────┘      └──────────────────────┘
```

Standard Protocol Approvals, Registrations, and Patient Consents:

Written informed consent was obtained from all participants. Ethical approval was received from the University of New South Wales Human Research Ethics Committee[151]. This study is compliant with STROBE guidelines[358].

Data Availability Statement:

Anonymized data from the MAS are available on request.

Neuropsychological assessment:

Trained research psychologists administered a battery of tests, grouped into cognitive domains: attention and processing speed (Digit Symbol-Coding[297], Trail Making Test-A[298]), executive function (Controlled Oral Word Association Test[298], Trail Making Test-B[298], Benton Visual Retention Test[300]), language (Boston Naming Test – 30 items[301], Semantic Fluency (Animals)[298]), visuospatial (Block Design[302]), and memory (Logical Memory[299], Rey Auditory Verbal Learning Test[298]). At each wave of the study, raw test scores were transformed to z-scores using the Wave 1 mean and SD values of a healthy reference group - 723 MAS participants. Domain scores were calculated by averaging the z-scores of component tests. These composite scores were then standardized as z-scores, calculated using the means and SDs for the Wave 1 healthy reference sample. A global cognition score was calculated by averaging all domain scores and again, transforming to a z-score using the healthy reference sample. Diagnosis of dementia was made using DSM-IV[359] criteria at each wave by a multidisciplinary consensus panel consisting of old age psychiatrists, neuropsychiatrists and neuropsychologists[151].

Radiological examination:

All MRI scans were performed at a single wave, on the same machine, a Philips 3T Achieva Quasar Dual scanner. A 3D T1-weighted sequence (1×1×1 mm3, TR/TE=6.39/2.9 ms), a T2-weighted fluid attenuation inversion recovery (FLAIR) sequence (TR/TE/TI=10000/110/2800 ms; thickness 3.5 mm; 0.898×0.898 mm2) and a susceptibility weighted imaging sequence (parameters previously described[71]) were performed. All images were co-registered using Statistical Parametric Mapping version 5 (SPM5). Total WMH volume quantification was automated, using FLAIR and T1-

weighted images[148], and adjusted for total intra-cranial volume[40]. All images were analyzed using MRIcron version 15[360]. Lacunes and CMB were defined using STRIVE criteria[38].

Perivascular space rating scale:

The number of PVS were counted in a single axial slice in the BG and CSO respectively according to a recently published scale[329]. For full details of the scale development including psychometric properties, see the original paper[329]. In brief, PVS were counted on pre-defined axial slices, 2mm and 37mm superior to the anterior commissure. PVS were defined according to STRIVE[38] criteria, i.e., they have CSF signal intensity and "follow the course of penetrating vessels, appearing linear when viewed parallel to the course of a penetrating vessels or round or ovoid when imaged perpendicular to the course of the vessel"[38]. They were differentiated from lacunes by their lack of a hyperintense rim on FLAIR sequences and by size, lacunes usually being greater than 3mm in diameter. We included PVS of any diameter however, if they fulfilled other diagnostic criteria[341]. Figure 4.2 shows examples of severe BG and severe CSO PVS pathology.

Figure 4.2. Severe basal ganglia (panel A) and severe centrum semiovale perivascular space pathology (panel B). Arrow points to an example of a PVS in each region.
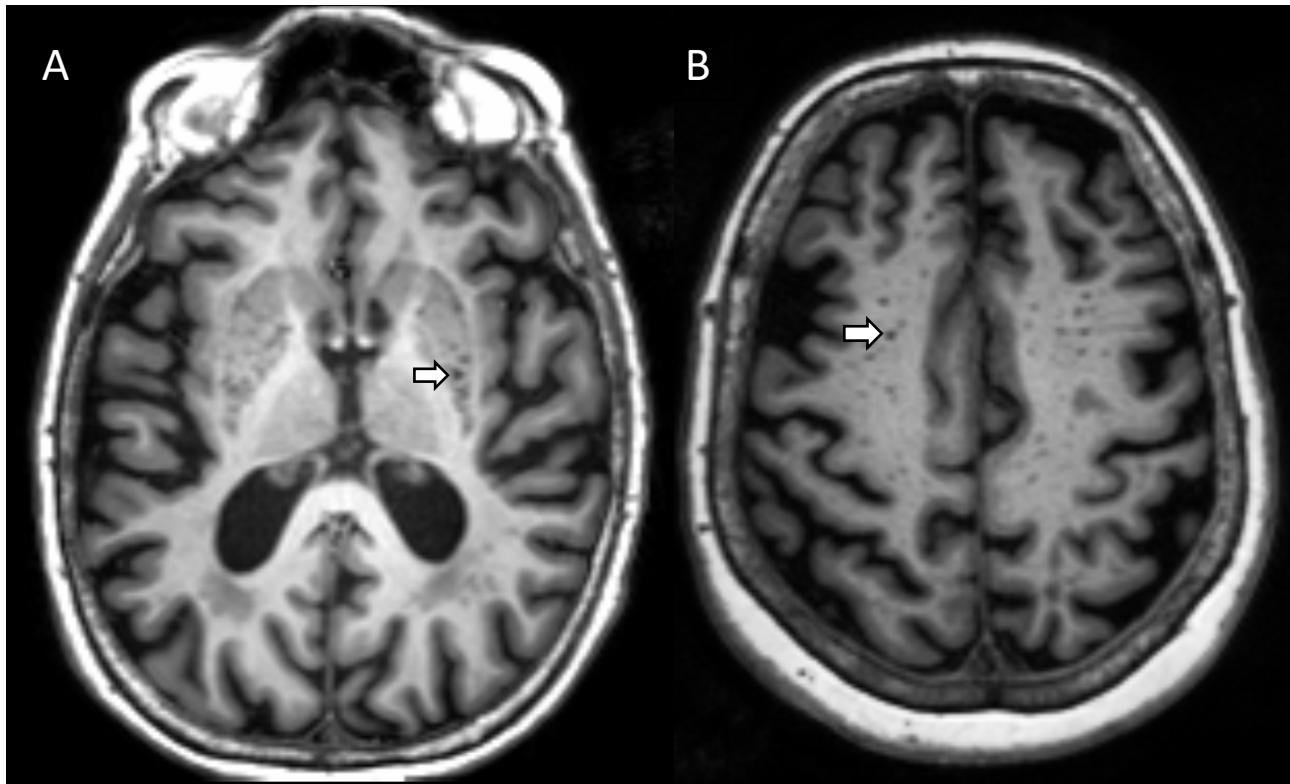
Covariates:

Hypertension was defined as a blood pressure of ≥140/90 mm/Hg (mean of two seated readings) or if the participant received a prior medical diagnosis of hypertension. Diabetic status was determined by a prior medical diagnosis or a fasting blood glucose value ≥7 mmol/L.

Analysis:

Based on the number of PVS in the BG slice, individuals were dichotomized at the top quartile, with those in the top quartile (≥7) considered to have severe pathology. This was repeated for the CSO slice, with individual with ≥2 PVS considered as having severe pathology. Four non-exclusive binary PVS predictor variables were then created for analysis; **i)** an "Any Severe PVS" group, representing those with severe PVS in either (or both) brain regions (severe BG *or* severe CSO PVS) vs. those with mild/absent (lower three quartiles) PVS in both regions**; ii)** a sub-sample of this group, the "Both Severe PVS", representing those with severe PVS in both regions of interest (severe BG *and* severe CSO PVS) vs. those with mild/absent (lower three quartiles) PVS in either region and: two regional subgroups; **iii)** a severe BG PVS group, with participants dichotomized into individuals with severe BG PVS pathology vs. those with mild/absent (lower three quartiles) BG PVS and **iv)** a severe CSO PVS pathology group, with participants dichotomized into individuals with severe CSO PVS pathology vs. those with mild/absent (lower three quartiles) CSO PVS.

The decision to examine those with the upper quartile of severity was made a-priori based on evidence suggesting there may only be cognitive sequelae for those the most severe PVS pathology, measured by size or frequency[61, 341].

To examine differences in baseline characteristics between groups defined by the four PVS binary predictors described above, t-test, Mann-Whitney U tests and χ^2-square tests were used.

WMH volume was log transformed to better approximate the normal distribution. Distributions of measures of cognition were inspected, and outliers (defined as being more than 3 standard deviations from the mean) were winsorised to values at that distance from mean values.

Linear mixed models (LMMs), with random intercepts and slopes, were used to examine relationships between PVS pathology in either region (Any Severe PVS group) vs mild/absent PVS in both region(s) and cognitive decline over three waves. Wave, PVS, and the PVS*wave product interaction term, were included in each of the equations along with the covariates listed below. Because we were interested in the effects of PVS on longitudinal cognitive decline, we report only parameter estimates (B-coefficients) with standard errors for the PVS*wave product interaction terms.

The following covariates were in the equation: Model 1 – age, sex, education, APOE-E4 carrier status, BMI, smoking status, hypertension, and diabetes. Model 2 included the covariates in Model 1 with the addition of the neuroimaging measures; WMH volume (log-transformed), presence of lacunes, presence of multiple (≥1) microbleeds. These analyses were then repeated for the other predictor variables: the Both Severe PVS, severe BG PVS and severe CSO PVS groups. Results from the LMM individual cognitive domain analyses were Bonferroni corrected for multiple testing(α set at 0.01). This considered each set of repeated analyses with the cognitive domains as outcomes as a family of five independent tests.

The associations of Any Severe PVS with incident dementia were examined using logistic regression to implement discrete time survival analysis[361-363]. This approach better handles events occurring in discrete time periods and provides flexible solutions to model data that violates the proportional hazard assumption[361]. We were interested in examining incident dementia from Wave 2 onwards and so only those not demented at Wave 2 (baseline) were used in the analyses. Data were structured into a person-period format. Each participant could have up to four rows of data, corresponding to the four assessment waves (Wave 3 to Wave 6). First, a dummy variable was created to indicate whether a participant developed dementia at a particular wave (0 = no dementia, 1 = dementia). Second, four discrete time indicators were computed by coding each of the four measurement waves, with a value of 1 being set for the time period it represents and all other values being set to 0. Finally, at a particular wave, if a participant was diagnosed with dementia, or if they dropped out (i.e., were censored), subsequent rows for that participant were removed. The four time indicators were included as predictors of dementia status in the logistic regression model while the

intercept was excluded. These variables were used as multiple intercepts to estimate the hazard function, or probability of the onset of dementia occurring in the period since the previous wave[361]. Importantly, the discrete time hazard is a conditional probability that denotes the event (incident dementia) will occur in a particular period, given it has not occurred earlier, and it is modelled as (log) odds in logistic regression. Regression equations are presented in Supplementary Appendix.

To determine whether severe BG or CSO PVS was associated with higher overall odds of dementia over all time periods relative to no severe PVS, PVS was included as a predictor in the analysis (proportional odds model). The analysis was repeated for the other PVS subtypes (i.e., Both severe PVS, severe BG PVS, severe CSO PVS). The regression coefficients of PVS were converted to odds ratios, and thus reflected the ratio of the odds of developing dementia for those with severe PVS (and subtypes) compared to those with a less severe pathology at every time period. ORs above and below 1 indicated that being in the PVS group was associated with higher or lower odds of developing dementia over time, respectively.

The above analysis was repeated to test whether the effect of PVS on dementia risk was moderated by time, including interaction terms between PVS and each of the four dummy variables and excluding the PVS term from the analysis (nonproportional odds model). Each interaction term represented the effect of PVS on dementia risk in each 2-year time period among those who were not demented at the previous wave. Hereafter, at year 4 corresponds to the 2-year period between year 2 and year 4, for example. In other words, each odd ratio corresponding to the interaction term indicated whether being in the severe PVS group, relative to the no severe PVS group, was associated with higher or lower odds of developing dementia over a 2-year period. Both sets of analyses adjusted for the same set of covariates as the LMM above.

All analyses were performed using SPSS statistical package (IBM SPSS Statistics for Windows, Version 25).

Missing data:

Both LMM and the discrete time survival approach better allow analysis of missing dependent variable data for a particular wave, compared with using the more

traditional case-wise deletion method, as they minimised loss of information, loss of power and biased estimates[364, 365]. Chained equations were used to impute missing covariate data - 25 imputations were chosen with parameter estimates based on the pooled estimates across all imputations.

To explore our patterns of missing data, we analysed potential differences in baseline characteristics between completers and non-completers. We also performed sensitivity analysis, repeating the main analyses for only those individuals with complete Global Cognition data over 4 years and complete dementia data over 8 years. We also tested the association between PVS and incident dementia by simulating data that assume higher rates of developing dementia among non-completers. This was done to increase confidence in our main results that assume data missing-at-random, given that is possible that attrition is higher for those more cognitively impaired (missing-not-at-random).

Finally, we conducted supplemental analyses, examining i) the association between PVS and decline in Global Cognition for the subset of 400 participants who were dementia free at baseline and; ii) the association of PVS with individual cognitive test results.

Results

Participant characteristics are presented in Table 4.1 (N=414; mean age 79.8, [SD 4.6]; 196 [47%] men). There were 157 (38%) individuals with severe PVS pathology in either region, 32 (7%) with severe pathology in both regions, 90 (22%) with severe BG PVS pathology and 99 (24%) with severe CSO PVS pathology.

Table 4.1. Characteristics of the study population (N=414).

Variable	Total	Any severe PVS (severe BG *or* CSO PVS pathology)			Both severe PVS (severe BG *and* CSO PVS pathology)			Basal ganglia PVS			Centrum semiovale PVS		
	N (%) or Mean ± SD	Mild/ absent N (%) or Mean ± SD	Severe N (%) or Mean ± SD	Difference	Mild/ absent N (%) or Mean ± SD	Severe N (%) or Mean ± SD	Difference	Mild/ absent N (%) or Mean ± SD	Severe N (%) or Mean ± SD	Difference	Mild/ absent N (%) or Mean ± SD	Severe N (%) or Mean ± SD	Difference
Total	414	257 (62)	157 (38)	-	382 (93)	32 (7)		324 (78)	90 (22)	-	315 (76)	99 (24)	-
Age, years	79.8 ± 4.6	79.7 ± 4.7	79.9 ± 4.5	t = -0.59, p=0.55	79.8 ± 4.7	79.6 ± 3.3	t = 0.21, p=0.83	79.7 4.7	80.1 4.4	t = -0.77, p=0.44	79.8 4.7	79.7 4.4	t = 0.20, p=0.84
Men	196 (47)	121(47)	75(48)	χ^2=0.02, p= 0.89	180(47)	16(50)	χ^2=0.10, p= 0.75	147 (45)	49 (54)	χ^2=2.33, p= 0.13	154 (49)	42 (42)	χ^2=1.26, p= 0.26
Education, years	11.9 ± 3.7	11.8 ± 3.7	12.0 ± 3.5	t=- -0.51, p=0.61	11.9 ± 3.6	12.2 ± 3.9	t=- -0.41, p=0.68	11.8 ± 3.6	12.3 ± 3.9	t= -1.24, p=0.22	11.9 ± 3.7	11.8 ± 3.4	t= -1.56, p=0.12

MMSE	Median = 29, IQR = 2	Median= 29 Mean rank= 209	Median= 29 Mean rank= 205	U=19,820, z=-0.31, p=0.76	Median= 29 Mean rank= 207	Median= 29 Mean rank= 211	U=6,236, z=-0.20, p=0.85	Median= 29 Mean rank= 209	Median= 29 Mean rank= 204	U=14,234, z=-0.35, p=0.72	Median= 29 Mean rank=207	Median= 29 Mean rank=209	U=15,709, z=-0.11, p=0.91
BMI[a]	26.9 ± 4.0	27.1 ± 4.2	26.6 ± 3.9	t = 1.16, p=0.25	26.9 ± 4.0	26.3 ±4.3	t =0.81, p=0.42	26.9 ± 4.1	26.7 ± 3.9	t = 0.46, p=0.65	27.0 4.1	26.4 4.0	t = 1.39, p=0.17
Smoker (in last month)[b]	19 (5)	15(6)	12(8)	χ^2 = 0.55, p = 0.46	25(7)	2(6)	χ2=0.01. Fishers p = 1.00	21(7)	6(7)	χ^2 <0.01, p = 0.98	19(6)	8(8)	χ^2 = 0.60, P = 0.44
Hypertension[c]	264 (64)	200(78)	129 (82)	χ2=0.84, p= 0.36	200(78)	27 (84)	χ2=0.44, p= 0.51	255(79)	74 (82)	χ2=0.40, p= 0.53	247(79)	82 (83)	χ2=0.72, p= 0.40
Diabetes[d]	81 (20)	52(20)	29(19)	χ^2=0.21, p=0.65	77(20)	4(13)	χ^2=1.11. Fisher's, p=0.36	65 (20)	16(18)	χ^2=0.25, p=0.62	64 (20)	17(17)	χ^2=0.49, p=0.48
APOE-ε4 carrier[e]	95 (23)	66(26)	29(19)	χ2=3.01, p=0.08	90(24)	5(16)	χ2=1.08, p=0.30	75 (23)	20 (22)	χ2=0.05, p=0.83	**81 (26)**	**14 (14)**	**χ2=5.84, p=0.02**

Total WMH volume mm^3	15,319 ± 14,296	14,556 ± 14,381	16,569 ± 14,111	t = -1.83, p= 0.07	14,934 ± 14,366	19,920 ± 12,751	t = -1.90, p= 0.06	**14,524 ± 14,427**	**18,181 ± 13,505**	**t = -2.89, p= 0.004**	15,047 ± 14,313	16186 ± 14279	t = -1.01 p= 0.31
Presence of lacunes	35 (9)	**15(6)**	**20(13)**	**χ^2 =6.00, p=0.01**	**28(7)**	**7(22)**	**χ^2 =8.07, p=0.004**	**20 (6)**	**15(17)**	**χ^2 = 0.02, p=0.002**	32 (7)	12(12)	χ^2 = 2.26, p=0.13
Presence of multiple CMB^f	80 (22)	**19(10)**	**20(17)**	**χ^2 = 3.89, p=0.048**	**34(11)**	**5(28)**	**χ^2 = 4.27, p=0.039**	**24 (9)**	**15(23)**	**χ^2 = 9.38 p=0.002**	29 (12)	10(14)	χ^2 = 0.24 p=0.63

Abbreviations: SD, Standard Deviation; MMSE, Mini Mental State Examination; BMI, Body Mass Index; WMH, White Matter Hyperintensity; CMB, cerebral microbleeds.

Bold text indicates p<0.05.

Data are missing for [a] 10 [b] 6 [c] 2 [d] 1 [e] 2 [f] 95 participants respectively.

The groups did not differ by age, sex or vascular risk factors, with the exception of those with severe CSO PVS who were less likely to be an APOE-ε4 carrier (14% compared to 26% with absent/mild CSO PVS, χ^2=5.84, df(1), p=0.02) Individuals with severe BG PVS had greater WMH volume (log transformed; t = -2.89, p=0.004), and were more likely to have lacunes (17% compared to 6% with absent/mild BG PVS, $\chi2$=10.02, df(1), p=0.002) and multiple CMB (23% compared to 9% with absent/mild BG PVS, $\chi2$= 9.38, df(1), p=0.002). In contrast, individuals with severe CSO PVS did not have greater amounts of other SVD imaging pathology.

Participants in the Any severe PVS group did not have a more rapid decline in global cognition compared to those with less severe disease (Table 4.2). Participants with severe disease in both locations however, had a more rapid decline in global cognition compared to those with less severe disease. This remained significant after adjusting for all demographic, vascular risk factors and other neuroimaging measures: Model 2 (Unstandardized B = -0.175, SE = 0.076, p= 0.020). When regional subgroups were examined, those with severe CSO PVS had a more rapid decline in global cognition compared to those with less severe disease, even after adjusting for all covariates: Model 2 (Unstandardized B = -0.111, SE = 0.048, p= 0.020).

Table 4.2. Longitudinal relationship of basal ganglia (BG) and centrum semiovale (CSO) perivascular spaces (PVS) with cognition over four years. (N=414); parameter estimate - unstandardized (SE) for the PVS*wave interaction term.

Cognition	Any severe PVS (Severe BG *or* severe CSO PVS)		Both severe PVS (Severe BG *and* severe CSO PVS)		Severe BG PVS (dichotomized around top quartile)		Severe CSO PVS (dichotomized around top quartile)	
	Model 1 B (SE), p-value	Model 2 B (SE), p-value	Model 1 B (SE), p-value	Model 2 B (SE), p-value	Model 1 B (SE), p-value	Model 2 B (SE), p-value	Model 1 B (SE), p-value	Model 2 B (SE), p-value
Global Cognition [a]	-0.057 (0.042), 0.182	-0.057 (0.042), 0.180	**-0.175 (0.076), 0.022**	**-0.175 (0.076), 0.020**	-0.034 (0.050), 0.492	-0.034 (0.050), 0.492	**-0.111 (0.048), 0.021**	**-0.111 (0.048), 0.020**
Cognitive Domain								
- Attention and Processing Speed [b]	-0.031 (0.050), 0.533	-0.031 (0.050), 0.536	-0.181 (0.092), 0.114	-0.182 (0.092), 0.047	-0.074 (0.059), 0.213	-0.073 (0.059), 0.213	-0.041 (0.057), 0.466	-0.041 (0.057), 0.468
- Executive function [c]	-0.044 (0.051), 0.383	-0.043 (0.051), 0.393	-0.164 (0.094), 0.081	-0.166 (0.094), 0.077	-0.089 (0.060), 0.140	-0.088 (0.060), 0.143	-0.038 (0.058), 0.504	-0.038 (0.058), 0.506
- Language [d]	-0.042 (0.040), 0.285	-0.042 (0.040), 0.295	-0.142 (0.072), 0.053	-0.142 (0.071), 0.047	-0.061 (0.047), 0.192	-0.060 (0.047), 0.201	-0.054 (0.045), 0.321	-0.054 (0.045), 0.181
- Visuospatial function [e]	-0.039 (0.045), 0.387	-0.039 (0.045), 0.387	-0.103 (0.080), 0.211	-0.103 (0.080), 0.195	0.000 (0.052), 0.998	0.000 (0.052), 0.997	-0.092 (0.050), 0.069	-0.092 (0.050), 0.069
- Memory [f]	-0.004 (0.040), 0.926	-0.003 (0.040), 0.934	-0.118 (0.072), 0.219	-0.117 (0.072), 0.102	0.019 (0.047), 0.683	0.020 (0.047), 0.673	-0.069 (0.045), 0.189	-0.070 (0.045), 0.118

Model 1 - adjusted for age, sex, education, APOE-E4 carrier status, BMI, smoking status, hypertension and diabetes.

Model 2 - adjusted for the covariates in Model 1 and WMH volume (log-transformed), presence of lacunes, presence of multiple (≥1) microbleeds.

Bold text indicates significance level of $p < 0.05$ for Global Cognition and $p < 0.01$ for the cognitive domain analysis (Bonferroni correction applied).

Data are missing on two or three of the assessment waves for [a] 35 [b] 40 [c] 59 [d] 26 [e] 34 [f] 30 participants respectively.

Results of the association of PVS with individual cognitive domains are presented in Table 4.2. The presence of severe total or regional BG or CSO PVS pathology was not associated with longitudinal decline in any of the five cognitive domains assessed after Bonferroni correction for multiple testing.

Ten participants were diagnosed with dementia at study baseline and four participants had missing dementia information from all waves. These 14 participants were excluded from the dementia analyses. Of 400 participants at baseline, 97 (24%) were diagnosed with dementia over the eight years of follow up. Of those with severe PVS in either region, 40 of 152 participants (26%) developed dementia and in individuals with severe PVS in both regions, 12 of 31 participants (39%) developed dementia. 27 of 89 (30%) participants with severe BG PVS at baseline, developed dementia, as did 25 of 94 participants (27%) with severe CSO PVS (Supplemental Table 4.4).

Table 4.3 displays the results of the discrete-time survival analysis. If a participant was in the Any severe PVS group or had just severe BG or CSO PVS pathology, they were no more likely to develop dementia, compared to an individual with mild/absent PVS pathology respectively. However, if they had pathology in both areas, they had nearly triple the odds of developing dementia, at every time period, over eight years (OR 2.91, 95% CI (1.43 – 5.95), p = 0.003), independent of other vascular and neuroimaging covariates.

Table 4.3. Longitudinal relationship of basal ganglia (BG) and centrum semiovale (CSO) perivascular spaces (PVS) with incident dementia over eight years. (N=400). Odds ratios of severe vs mild/absent (upper vs lower three quartiles) PVS pathology.

	Any severe PVS (Severe BG *or* severe CSO PVS)		Both severe PVS (Severe BG *and* severe CSO PVS)		Severe BG PVS (dichotomized around top quartile)		Severe CSO PVS (dichotomized around top quartile)	
	Model 1 OR (95% CI), p-value	**Model 2** OR (95% CI), p-value	**Model 1** OR (95% CI), p-value	**Model 2** OR (95% CI), p-value	**Model 1** OR (95% CI), p-value	**Model 2** OR (95% CI), p-value	**Model 1** OR (95% CI), p-value	**Model 2** OR (95% CI), p-value
Overall (across all waves)	1.32 (0.84 - 2.08), 0.232	1.21 (0.76 - 1.93), 0.425	**3.18 (1.59 - 6.38), 0.001**	**2.91 (1.43 - 5.95), 0.003**	1.63 (0.99 - 2.70), 0.056	1.46 (0.86 - 2.46), 0.157	1.46 (0.87 - 2.44), 0.150	1.42 (0.84 - 2.38), 0.190
Year 2	1.02 (0.37 - 2.79), 0.968	0.92 (0.34 - 2.51), 0.876	2.96 (0.83 - 10.56), 0.110	2.70 (0.71 - 10.31), 0.145	0.90 (0.63 - 1.29), 0.860	0.81 (0.25 - 2.62), 0.724	1.93 (0.69 - 5.42), 0.210	1.83 (0.65 - 5.18), 0.253
Year 4	**2.76 (1.13 - 6.70), 0.025**	**2.48 (1.00 - 6.11), 0.049**	**5.35 (1.73 - 16.53), 0.004**	**4.75 (1.51 - 14.95), 0.008**	2.31 (0.96 - 5.56), 0.069	2.05 (0.81 - 5.17), 0.129	**3.31 (1.35 - 8.12), 0.009**	**3.12 (1.26 - 7.73), 0.014**
Year 6	2.20 (0.80 - 6.05), 0.127	2.07 (0.75 - 5.73), 0.162	**5.84 (1.42 - 24.05), 0.015**	**5.44 (1.31 - 22.61), 0.020**	**3.25 (1.14 - 9.30), 0.028**	**2.93 (1.01 - 8.45), 0.047**	1.77 (0.58 - 5.39), 0.315	1.78 (0.58 - 5.45), 0.309
Year 8	0.61 (0.26 - 1.42), 0.253	0.57 (0.24 - 1.34), 0.196	0.64 (0.08 - 5.28), 0.675	0.61 (0.07 - 5.08), 0.646	1.04 (0.38 - 2.84), 0.942	0.93 (0.34 - 2.56), 0.884	0.42 (0.14 - 1.28), 0.126	0.41 (0.13 - 1.28), 0.125

Model 1 - adjusted for age, sex, education, APOE-E4 carrier status, BMI, smoking status, hypertension, diabetes.

Model 2 - adjusted for the covariates in Model 1 and WMH volume (log-transformed), presence of lacunes, presence of multiple (≥1) microbleeds.

Bold text indicates p<0.05.

There was a strong interaction with time, such that for all four predictor variable groups, severe PVS was associated with an increased risk of dementia at either year 4 or 6. This effect remained after adjusting for all other covariates. The strongest effect was seen in those with severe pathology in both areas, with an OR of 4.75, 95% CI (1.51 - 14.95), p = 0.008 of developing dementia at year 4 compared to those with absent/mild PVS in either region. The association of severe PVS with dementia was not seen at year 8. Figure 4.3 shows, for each wave, the predicted probability of developing dementia in each PVS group, based on the discrete-time survival analysis results. The full results (Model 2 only) are presented in Supplemental Table 4.5 so the odds ratios of the other covariates in the model can be seen, with age and APOE-E4 carrier status having the strongest relationship with dementia status.

Figure 4.3. Predicted probability at each wave of developing dementia since baseline in each PVS group, based on discrete time survival analysis.
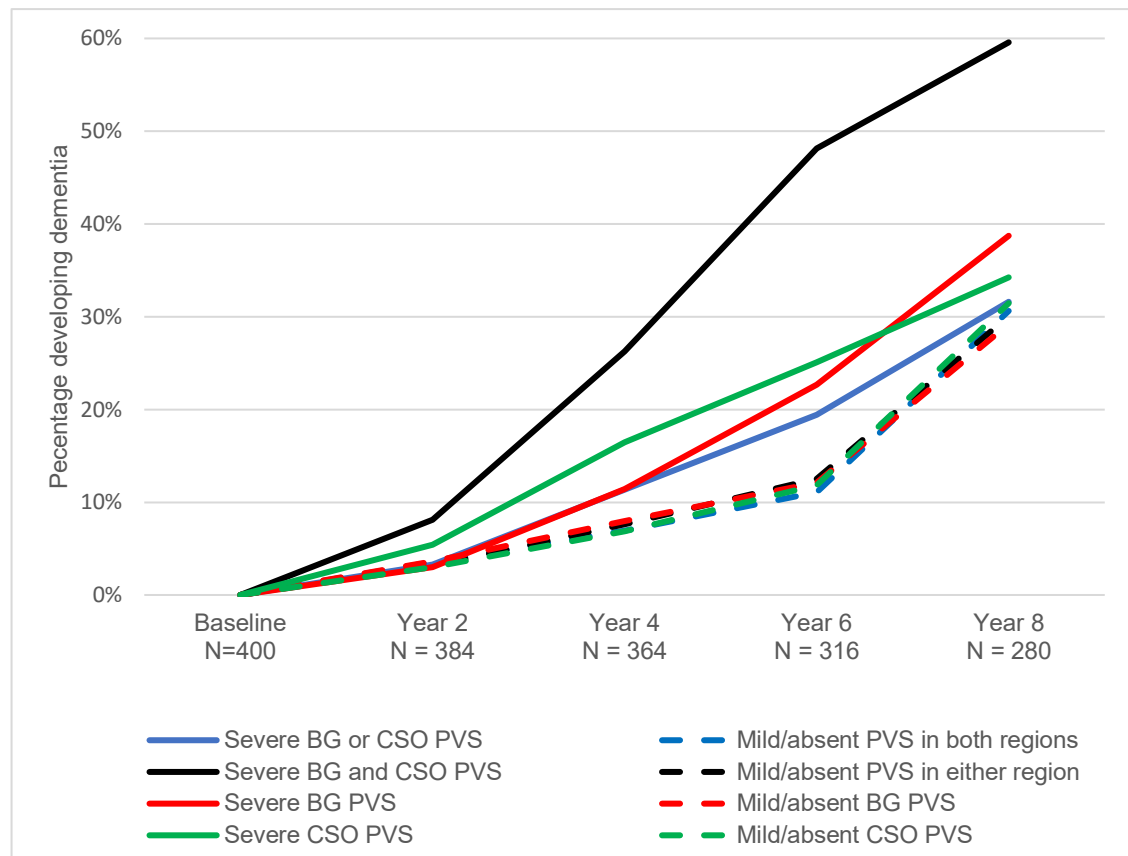
PVS: Perivascular spaces. BG: basal ganglia. CSO: centrum semiovale.

Predicted values adjusted for age, sex, education, APOE-E4 carrier status, BMI, smoking status, hypertension, diabetes, WMH volume (log-transformed), presence of lacunes, presence of multiple (≥1) microbleeds.

A comparison of the baseline characteristics for completers vs. non-completers is shown in Supplemental Table 4.6. Non-completers were significantly older (80.6 years vs 79.3 years, t=2.75, p=0.006), more likely to be male (60% vs 43%, χ2=9.89, p= 0.002) and have lacunes (13% vs 6%, χ2=5.15, p= 0.02).

Results from the sensitivity analyses examining only those 331 participants who had full Global Cognition data for all 4 years are presented in Supplemental Table 4.7. Similar to the original analysis, greater decline in global cognition is seen for those with severe CSO PVS and those with severe PVS in both regions. Life tables for the dementia analysis are presented in Supplemental Table 4.4 with complete data available on 280 participants (70% completers) by year 8. The results of the completers-only dementia analysis (Supplemental Table 4.8) resemble the main analysis, with participants with severe PVS pathology in both regions demonstrating an overall increase in dementia incidence. When time interactions were examined, the association between PVS pathology in either location and mid-study dementia incidence (year 4-6) remained. To investigate whether the non-significant time interaction effects at year 8 were partly due to attrition bias driven by dementia status, we simulated incident dementia data for non-completers that assumed higher rates of developing dementia (Supplemental Table 4.9). This did not significantly change the overall results. Supplemental Table 4.10 displays the results of the LMM analysis for the subset of 400 participants who were dementia-free at the start of this study, with similar results to the original cohort of 414 participants. Supplemental Table 4.11 presents the associations of PVS with individual cognitive test scores; participants with severe pathology in both regions show impairments on tests associated with verbal learning, attention and processing speed and language.

Discussion

We found that having severe PVS pathology in both regions examined predicted incident dementia. When regions were assessed individually, severe BG PVS and CSO PVS pathology predicted early dementia at years six and four, respectively. There were also regional differences when assessing longitudinal cognitive change. It was also only

participants with severe PVS pathology in both regions, or those with severe CSO PVS pathology, that had an increased rate of cognitive decline over four years. Severe BG PVS pathology was not associated with cognitive decline.

These results remained significant after adjusting for the other neuroimaging measures of SVD, suggesting there is an independent mechanism for PVS as a biomarker of cognitive impairment and dementia apart from being a general marker of SVD pathology. Dilated PVS may be a biomarker of impaired waste clearance[332, 333, 366] for example.

The effect of PVS may differ by region. For CSO PVS specifically, several non-exclusive mechanisms may impact on cognition. Enlargement of PVS in the white matter is reportedly associated with cerebral amyloid-β pathologies[357] , and CSO PVS pathology might indicate the presence of CAA or a mixed hypertensive / CAA[63, 346, 347, 354] pathophysiology. It may be a more diffuse CAA-Alzheimer's association driving cognitive sequelae, with a vicious cycle of impaired clearance of amyloid and other toxins, leading to greater damage of the neurovascular unit and cerebral parenchyma. Alternatively, it may be damage to particular WM tracts in the CSO, including through CAA pathology that leads to cognitive impairment.

In contrast, BG PVS are a marker for hypertensive arteriopathy, as are other traditional markers such as lacunes, WMH and multiple CMB, albeit with some regional variation[71]. This would explain the associations between BG PVS and other neuroimaging measures, which did not occur with CSO PVS.

The strongest findings were for those 32 (7%) participants with severe PVS in both regions. This could be due to two non-exclusive mechanisms. First, that this group has the most severe and widespread PVS pathology. Post-hoc analysis found this group did not have a significant increase in PVS frequency, with a median of 10 BG PVS and four CSO PVS, compared to nine BG PVS in those with just severe BG PVS pathology and four CSO PVS in those with just severe CSO PVS pathology. Alternatively, or additionally, there may be an additive effect of the two different pathologies present in the different regions – hypertensive arteriopathy and CAA, which together produce a larger association with cognitive decline and dementia.

The weak association between PVS and vascular risk factors is unsurprising given the literature, which reports variable results for the association of PVS with both vascular and demographic risk factors[62, 329, 337, 341, 345, 346, 348]. This may be partially explained by the wide variety of definitions and rating scales used to quantify PVS.

We found associations with decline in global cognition but not in any of the constituent cognitive domains examined. Some of these domains had a trend toward significance and we can speculate there may be an additive effect, that when combined into a global cognition composite produced a significant association. The majority of studies of PVS and cognition are cross-sectional with two recent meta-analyses reporting no association of PVS severity with impaired cognition[327, 328]. We could find only three prospective studies examining cognitive change. Ding et al.,[341] reported associations of large PVS with declines in processing speed independent of other SVD markers, but not of verbal memory or executive function. Zhu et al.,[61] reported that BG PVS were associated with declines in Trails-A and B, but WM PVS were not. They did not adjust for the presence of other SVD neuroimaging measures. After adjusting for WMH and CMB, Benjamin et al.,[160] did not find an association between PVS and decline in total cognition, executive function or processing speed. The mixed results suggest insufficient evidence linking either total or regional PVS and declines in specific cognitive domains.

For incident dementia, our results are somewhat consistent with the two longitudinal studies of PVS and dementia in the general population. Ding et al.,[341] reported an association between large PVS and vascular but not all-type dementia or AD. Their mean follow-up of 5.2 years is similar to the period in which we found the strongest effect of PVS. Similarly, Zhu et al.[61] had a median follow-up of 3.5 years in their study which found an association between WM PVS and dementia, after adjusting for WMH.

When examining regions individually, the lack of association between BG and CSO PVS and dementia across eight years may reflect the sharp increase in the numbers developing dementia without severe PVS pathology at the year 8 follow up, when participants were, on average, in their late 80s. New dementia cases peaked at year 4 in those with severe CSO PVS pathology and then declined at subsequent waves, but for those with mild/absent PVS pathology, new cases continued to rise thorough the

eight years. Those with severe PVS pathology may have dropped out earlier and the signal from PVS was lost due to the stronger effect of age over time. The sensitivity analysis showed non-completers were more likely to be older and male, both groups which have increased morbidity and mortality, supporting this argument.

Unexpectedly, individuals with severe CSO PVS pathology were *less* likely to be an APOE-ε4 carrier. The association between APOE genotype and PVS has been little studied with one recent paper finding no association[367]. Our seemingly paradoxical finding is not easily explained and may be a type 1 error and affected by sample size (i.e., only 14 individuals had severe CSO PVS pathology and were APOE-ε4 carriers).

There were limitations to our study. Although eight years is a longer follow-up period than most comparable studies, even longer prospective periods may be more informative for dementia analysis. We also only had detailed cognitive data for four years. The scale used to rate PVS had good psychometric properties and was easy to use[329], but the selection of two pre-determined slices may have missed PVS in other areas, particularly for those with an atypical pattern of PVS distribution. The scale rated PVS primarily from T1-weighted images, but PVS are more easily visible on T2-weighted imaging[187, 329]. The scale may therefore have underestimated PVS frequency. There was attrition, which increased over the study period and by year eight, dementia status could not be determined in 30% of participants. Using LMM and discrete-time survival analysis logistic regression minimized data loss and maximized power as we could still include participants who did not have complete data across all waves. Results were unaffected when sensitivity analysis was used to examine only study completers. These analytic strategies assume that missing is at random or completely at random. Indeed, our attrition data are consistent with other ageing and dementia longitudinal studies[368], and non-completers are generally more likely to be unwell or cognitively impaired[368, 369]. Our analysis confirmed that non-completers had characteristics at baseline (age, male sex, presence of lacunes) that predisposed them to greater morbidity. The potential informative attrition may have biased our results, especially the surprising finding on dementia risk at year eight. Our sensitivity analysis suggested that informative attribution could not fully explain the current results however, which requires further investigation.

Conclusions

We found differential associations between the region of PVS pathology and cognitive decline and incident dementia. This suggests differences in their underlying pathology and/or differential impacts due to the location of PVS and effect on white matter tracts. The associations remained significant in the presence of other SVD neuroimaging markers, suggesting at least a partially independent pathway of PVS related injury. Further research is needed into the aetiology and sequelae of PVS pathology since PVS may be an important potential biomarker to help with early dementia diagnosis, prognosis and subtyping. Importantly, future studies should divide PVS analyses by region and attempt to standardize PVS visual rating. Study duration needs to be carefully considered when examining incident dementia due to the interaction of time with PVS.

Supplemental Data

Supplementary Appendix

Equation for discrete-time hazard model examining the overall effect of PVS after adjusting for covariates

logit $h(t_{ij})$ = [$\alpha_1 D_{1ij} + \alpha_2 D_{2ij} + \alpha_3 D_{3ij} + \alpha_4 D_{4ij}$] + $\beta_1 PVS_i + \beta_2 Age_i + \beta_3 Sex_i + \beta_4 Education_i + \beta_5 APOE\text{-}\varepsilon 4_i + \beta_6 BMI_i + \beta_7 Smoking_i + \beta_8 Hypertension_i + \beta_9 Diabetes_i$

Where,

$h(t)$ = discrete-time hazard

individual i = 1,2, . . . , n

time period j = 1,2,3,4

α = intercept parameter

D = time indicator

β = slope parameter

Equation for discrete-time hazard model examining the time-varying effect of PVS after adjusting for covariates

logit $h(t_{ij})$ = [$\alpha_1 D_{1ij} + \alpha_2 D_{2ij} + \alpha_3 D_{3ij} + \alpha_4 D_{4ij}$] + $\beta_1 PVS_i + \beta_2 Age_i + \beta_3 Sex_i + \beta_4 Education_i + \beta_5 APOE\text{-}\varepsilon 4_i + \beta_6 BMI_i + \beta_7 Smoking_i + \beta_8 Hypertension_i + \beta_9 Diabetes_i + \beta_{10}(D_{1ij} \times PVS_i) + \beta_{11}(D_{2ij} \times PVS_i) + \beta_{12}(D_{3ij} \times PVS_i) + \beta_{13}(D_{4ij} \times PVS_i)$

Supplemental Table 4.4: Life table - cumulative dementia status and missing data at each wave.

		Baseline – Year 0	Year 2	Year 4	Year 6	Year 8
Severe BG PVS	**- dementia**	0	4 (5%)	13 (16%)	20 (30%)	27 (46%)
	- no dementia	89 (100%)	81 (95%)	68 (84%)	47 (70%)	32 (54%)
Mild/absent BG PVS pathology	**- dementia**	0	14 (5%)	28 (10%)	38 (15%)	70 (32%)
	- no dementia	311 (100%)	285 (95%)	255 (90%)	211 (85%)	151 (68%)
Severe CSO PVS	**- dementia**	0	6 (6%)	16 (18%)	21 (27%)	25 (34%)
	- no dementia	94 (100%)	87 (94%)	73 (82%)	58 (73%)	49 (66%)
Mild/absent CSO PVS pathology	**- dementia**	0	12 (4%)	25 (9%)	37 (16%)	72 (35%)
	- no dementia	306 (100%)	279 (96%)	250 (91%)	200 (84%)	134 (65%)
Dementia		0	18 (4.7%)	41 (11.3%)	58 (18.4%)	97 (34.6%)
No-dementia		400	366 (95%)	323 (89%)	258 (82%)	183 (65%)
Sub-total		400	384	364	316	280
Missing (cumulative)		0	16	36	84	120
Total		**400**	**400**	**400**	**400**	**400**

Supplemental Table 4.5: Longitudinal relationship of basal ganglia (BG) and centrum semiovale (CSO) perivascular spaces (PVS) with incident dementia over eight years (N=400). Odds ratios of severe vs mild/absent (upper vs lower three quartiles) PVS pathology and for all covariates in the model.

	Any severe PVS (Severe BG *or* severe CSO PVS)	Both severe PVS (Severe BG *and* severe CSO PVS)	Severe BG PVS (dichotomized around top quartile)	Severe CSO PVS (dichotomized around top quartile)
	Model 2 OR (95% CI), p-value	Model 2 OR (95% CI), p-value	Model 2 OR (95% CI), p-value	Model 2 OR (95% CI), p-value
PVS-time interaction at Year 2	0.92 (0.34 - 2.51), 0.876	2.70 (0.71 - 10.31), 0.145	0.81 (0.25 - 2.62), 0.724	1.83 (0.65 - 5.18), 0.253
PVS-time interaction at Year 4	2.48 (1.00 - 6.11), 0.049	4.75 (1.51 - 14.95), 0.008	2.05 (0.81 - 5.17), 0.129	3.12 (1.26 - 7.73), 0.014
PVS-time interaction at Year 6	2.07 (0.75 - 5.73), 0.162	5.44 (1.31 - 22.61), 0.020	2.93 (1.01 - 8.45), 0.047	1.78 (0.58 - 5.45), 0.309
PVS-time interaction at Year 8	0.57 (0.24 - 1.34), 0.196	0.61 (0.07 - 5.08), 0.646	0.93 (0.34 - 2.56), 0.884	0.41 (0.13 - 1.28), 0.125
Age	1.14 (1.08 - 1.20), 0.000	1.15 (1.09 - 1.20), 0.000	1.14 (1.08 - 1.20), 0.000	1.14 (1.09 - 1.20), 0.000
Sex, men	1.47 (0.91 - 2.35), 0.113	1.58 (0.98 - 2.54), 0.059	1.49 (0.93 - 2.39), 0.098	1.52 (0.95 - 2.44), 0.083
Education, years	0.94 (0.88 - 1.01), 0.077	0.94 (0.88 - 1.00), 0.063	0.94 (0.88 - 1.01), 0.075	0.94 (0.88 - 1.00), 0.066
APOE-ε4 carrier	1.86 (1.14 - 3.03), 0.013	1.90 (1.16 - 3.10), 0.010	1.82 (1.12 - 2.95), 0.016	1.91 (1.16 - 3.15), 0.011
BMI	1.01 (0.95 - 1.07), 0.780	1.01 (0.96 - 1.07), 0.667	1.01 (0.95 - 1.07), 0.756	1.01 (0.95 - 1.07), 0.671

Smoker (in last month)	1.39 (0.58 - 3.38), 0.462	1.36 (0.56 - 3.30), 0.502	1.44 (0.59 - 3.50), 0.422	1.36 (0.56 - 3.30), 0.495
Diabetes	1.30 (0.76 - 2.22), 0.336	1.34 (0.78 - 2.29), 0.287	1.31 (0.77 - 2.24), 0.325	1.32 (0.77 - 2.27), 0.305
Hypertension	0.84 (0.49 - 1.45), 0.532	0.77 (0.45 - 1.33), 0.351	0.85 (0.50 - 1.47), 0.570	0.79 (0.46 - 1.37), 0.409
WMH volume	1.38 (0.72 - 2.64), 0.334	1.35 (0.70 - 2.60), 0.372	1.37 (0.71 - 2.62), 0.344	1.36 (0.71 - 2.61), 0.361
Presence of lacunes	1.85 (0.88 - 3.91), 0.106	1.77 (0.85 - 3.68), 0.125	1.76 (0.84 - 3.72), 0.135	1.87 (0.89 - 3.92), 0.100
Presence of multiple CMB	1.08 (0.51 - 2.31), 0.835	1.00 (0.47 - 2.13), 0.991	1.08 (0.51 - 2.31), 0.843	1.11 (0.52 - 2.36), 0.788

Model 2 - adjusted for age, sex, education, APOE-E4 carrier status, BMI, smoking status, hypertension, diabetes, WMH volume (log-transformed), presence of lacunes, presence of multiple (≥1) microbleeds.

Supplemental Table 4.6. Differences in baseline characteristics between completers and non-completers at year 8.

Variable	Non-completer (n=120)	Completer (n=280)	
	Mild/absent N (%) or Mean ± SD	Severe N (%) or Mean ± SD	Difference
Age, years	80.6 ± 4.5	79.3 ± 4.5	**t = 2.749, p=0.006**
Men	72(60)	120(43)	**χ2=9.89, p= 0.002**
Education, years	12.1 ± 3.6	11.8 ± 3.6	t=0.967, p=0.33
MMSE	Median= 28 Mean rank= 195	Median= 29 Mean rank= 203	U=17,427, z=0.61, p=0.54
BMI[a]	26.4 ± 3.6	27.1 ± 4.2	t = -1.548, p=0.12
Smoker (in last month)	6(5)	21(8)	$\chi^2 = 0.88$, p = 0.35
Hypertension	101(84)	219 (79)	χ2=1.70, p= 0.19
Diabetes	23(19)	55(20)	χ^2=0.02, p=0.90
APOE-ε4 carrier	22(18)	70(25)	χ2=2.21, p=0.14
Total WMH volume mm^3	17,121 ± 15,639	14,549 ± 13,749	t = 1.644, p= 0.10
Presence of lacunes	16(13)	18(6)	**$\chi^2=$ 5.15, p=0.02**
Presence of multiple CMB	11(13)	27(12)	$\chi^2=$ 0.11, p=0.74

Abbreviations: SD, Standard Deviation; MMSE, Mini Mental State Examination; BMI, Body Mass Index; WMH, White Matter Hyperintensity; CMB, cerebral microbleeds.

Supplemental Table 4.7. Sensitivity analysis - longitudinal relationship of basal ganglia (BG) and centrum semiovale (CSO) perivascular spaces (PVS) with cognition, for participants with full data over four years (N=331); parameter estimate - unstandardized (SE) for the PVS*wave interaction term.

Cognition	Any severe PVS (Severe BG *or* severe CSO PVS)		Both severe PVS (Severe BG *and* severe CSO PVS)		Severe BG PVS (dichotomized around top quartile)		Severe CSO PVS (dichotomized around top quartile)	
	Model 1 B (SE), p-value	Model 2 B (SE), p-value	Model 1 B (SE), p-value	Model 2 B (SE), p-value	Model 1 B (SE), p-value	Model 2 B (SE), p-value	Model 1 B (SE), p-value	Model 2 B (SE), p-value
Global Cognition	-0.053 (0.042), 0.205	-0.053 (0.042), 0.205	**-0.199 (0.074), 0.007**	**-0.199 (0.074), 0.007**	-0.053 (0.049), 0.278	-0.053 (0.049), 0.278	**-0.098 (0.047), 0.037**	**-0.098 (0.047), 0.037**
Cognitive Domain								
- Attention and Processing Speed	-0.057 (0.051), - 0.269	-0.057 (0.051), 0.266	-0.211 (0.094), 0.025	-0.211 (0.094), 0.025	-0.087 (0.060), 0.275	-0.087 (0.060), 0.149	-0.073 (0.058), 0.839	-0.073 (0.058), 0.211
- Executive function	-0.050 (0.053), - 0.062	-0.048 (0.053), 0.358	-0.050 (0.099), 0.133	-0.158 (0.099), 0.111	-0.088 (0.063), 0.833	-0.089 (0.063), 0.157	-0.042 (0.060), 0.721	-0.041 (0.060), 0.492
- Language	-0.160 (0.074), 0.031	-0.039 (0.041), 0.341	-0.160 (0.074), 0.031	-0.160 (0.074), 0.031	-0.060 (0.048), 0.210	-0.060 (0.048), 0.210	-0.058 (0.047), 0.220	-0.058 (0.047), 0.220
- Visuospatial function	-0.115 (0.082), 1.000	-0.029 (0.046), 0.520	-0.115 (0.082), 1.000	-0.115 (0.082), 0.866	0.007 (0.054), 0.967	0.007 (0.054), 0.895	-0.091 (0.052), 0.080	-0.091 (0.052), 0.123
- Memory	-0.157 (0.076), 0.158	-0.013 (0.041), 0.762	-0.157 (0.076), 0.158	-0.157 (0.076), 0.040	-0.012 (0.049), 0.966	-0.012 (0.049), 0.807	-0.066 (0.048), 0.512	-0.066 (0.048), 0.187

Model 1 - adjusted for age, sex, education, APOE-E4 carrier status, BMI, smoking status, hypertension and diabetes.

Model 2 - adjusted for the covariates in Model 1 and WMH volume (log-transformed), presence of lacunes, presence of multiple (≥1) microbleeds.

Bold text indicates significance level of $p < 0.05$ for Global Cognition and $p < 0.01$ for the cognitive domain analysis (Bonferroni correction applied).

Supplemental Table 4.8: Sensitivity analysis - longitudinal relationship of basal ganglia (BG) and centrum semiovale (CSO) perivascular spaces (PVS) with incident dementia, for participants with known dementia status over eight years. (N=280). Odds ratios of severe vs mild/absent (upper vs lower three quartiles) PVS pathology.

	Any severe PVS (Severe BG *or* severe CSO PVS)		Both severe PVS (Severe BG *and* severe CSO PVS)		Severe BG PVS (dichotomized around top quartile)		Severe CSO PVS (dichotomized around top quartile)	
	Model 1 OR (95% CI), p-value	Model 2 OR (95% CI), p-value	Model 1 OR (95% CI), p-value	Model 2 OR (95% CI), p-value	Model 1 OR (95% CI), p-value	Model 2 OR (95% CI), p-value	Model 1 OR (95% CI), p-value	Model 2 OR (95% CI), p-value
Overall (across all waves)	1.26 (0.79 - 2.01), 0.324	1.08 (0.67 - 1.76), 0.744	**3.38 (1.65 - 6.93), 0.001**	**3.04 (1.47 - 6.32), 0.003**	**1.76 (1.05 - 2.96), 0.032**	1.50 (0.87 - 2.58), 0.143	1.31 (0.78 - 2.21), 0.309	1.23 (0.72 - 2.10), 0.442
Year 2	0.93 (0.34 - 2.53), 0.881	0.77 (0.27 - 2.15), 0.616	3.36 (0.84 - 13.45), 0.086	3.05 (0.76 - 12.21), 0.114	0.97 (0.30 - 3.13), 0.966	0.84 (0.25 - 2.79), 0.771	1.63 (0.57 - 4.66), 0.365	1.47 (0.50 - 4.30), 0.481
Year 4	2.67 (1.07 - 6.65), 0.035	2.25 (0.89 - 5.70), 0.088	**6.47 (1.97 - 21.29), 0.002**	**5.75 (1.73 - 19.13), 0.004**	**2.74 (1.07 - 7.01), 0.036**	2.37 (0.91 - 6.18), 0.078	**2.88 (1.15 - 7.21), 0.024**	**2.63 (1.03 - 6.70), 0.043**
Year 6	2.15 (0.77 - 6.00), 0.143	1.89 (0.67 - 5.36), 0.230	**5.39 (1.28 - 22.70), 0.022**	**4.81 (1.13 - 20.40), 0.033**	3.41 (1.16 - 9.99), 0.025	2.86 (0.96 - 8.58), 0.060	1.64 (0.53 - 5.06), 0.390	1.62 (0.52 - 5.02), 0.406
Year 8	0.60 (0.26 - 1.42), 0.246	0.54 (0.23 - 1.29), 0.164	0.63 (0.08 - 5.31), 0.673	0.58 (0.07 - 4.87), 0.613	1.07 (0.39 - 2.94), 0.898	0.90 (0.32 - 2.53), 0.847	0.40 (0.13 - 1.24), 0.112	0.39 (0.13 - 1.23), 0.108

Model 1 - adjusted for age, sex, education, APOE-E4 carrier status, BMI, smoking status, hypertension, diabetes.

Model 2 - adjusted for the covariates in Model 1 and WMH volume (log-transformed), presence of lacunes, presence of multiple (≥1) microbleeds.

Bold text indicates p<0.05.

Supplemental Table 4.9: Sensitivity analysis - longitudinal relationship of basal ganglia (BG) and centrum semiovale (CSO) perivascular spaces (PVS) with incident dementia at year 8, simulating increased proportions of incident dementia in non-completers.

Odds ratios and p-values of severe vs mild/absent (upper vs lower three quartiles) PVS pathology presented. Dementia data were randomly generated for non-completers that assumed the same (+0%) or higher rates of developing dementia (+10%, +20%, +30%) than completers. Discrete time survival analysis was then conducted for Model 2 using the simulated data. This procedure was repeated 10 times.

	Trial 1 OR, p-value	Trial 2 OR, p-value	Trial 3 OR, p-value	Trial 4 OR, p-value	Trial 5 OR, p-value	Trial 6 OR, p-value	Trial 7 OR, p-value	Trial 8 OR, p-value	Trial 9 OR, p-value	Trial 10 OR, p-value	Percentage of trials with p <0.05
Any severe PVS (Severe BG *or* severe CSO PVS)											
Same (+0%)	0.59, 0.084	0.68, 0.197	0.66, 0.177	0.5, 0.026	0.78, 0.379	0.95, 0.864	0.52, 0.032	0.66, 0.155	0.75, 0.325	0.81, 0.500	20%
+10%	0.68, 0.196	0.59, 0.06	0.81, 0.461	0.75, 0.312	0.56, 0.042	0.62, 0.103	0.54, 0.045	0.65, 0.143	0.90, 0.721	0.77, 0.351	20%
+20%	0.79, 0.394	0.64, 0.119	0.69, 0.171	0.51, 0.021	0.88, 0.644	0.67, 0.154	0.49, 0.016	0.76, 0.325	0.53, 0.032	0.59, 0.064	30%
+30%	1.05, 0.867	0.80, 0.411	0.89, 0.678	0.52, 0.020	0.71, 0.214	0.92, 0.760	0.89, 0.670	0.82, 0.476	0.72, 0.238	0.74, 0.282	10%

Both severe PVS

(Severe BG *and* severe CSO PVS)

Same (+0%)	0.70, 0.593	0.40, 0.239	0.99, 0.984	0.34, 0.171	1.41, 0.540	1.32, 0.656	0.75, 0.634	1.11, 0.864	0.54, 0.347	0.75, 0.666	0%
+10%	0.89, 0.856	0.58, 0.355	0.68, 0.524	1.19, 0.779	0.52, 0.322	0.86, 0.810	0.17, 0.088	1.56, 0.422	0.69, 0.544	0.68, 0.523	0%
+20%	1.15, 0.802	0.71, 0.580	1.32, 0.608	1.23, 0.735	0.94, 0.92	0.48, 0.271	0.51, 0.304	0.73, 0.595	0.76, 0.701	1.03, 0.957	0%
+30%	1.12, 0.841	0.82, 0.723	2.23, 0.111	0.33, 0.170	0.55, 0.369	1.42, 0.495	0.71, 0.575	0.26, 0.201	0.72, 0.590	0.71, 0.587	0%

Severe BG PVS

(dichotomized around top quartile)

Same (+0%)	0.79, 0.507	1.07, 0.845	1.13, 0.724	0.59, 0.146	1.28, 0.458	1.42, 0.291	0.72, 0.368	1.14, 0.703	1.29, 0.432	1.11, 0.767	0%
+10%	0.90, 0.769	0.84, 0.591	1.24, 0.517	1.15, 0.672	0.93, 0.824	0.93, 0.84	0.67, 0.286	1.36, 0.351	1.23, 0.527	0.96, 0.892	0%
+20%	1.27, 0.454	1.01, 0.987	1.01, 0.967	0.83, 0.578	1.64, 0.117	0.96, 0.896	0.66, 0.244	1.21, 0.547	0.79, 0.506	1.22, 0.541	0%
+30%	1.41, 0.305	1.27, 0.441	1.60, 0.137	0.77, 0.445	0.89, 0.724	1.50, 0.199	1.10, 0.780	1.18, 0.617	0.93, 0.818	1.42, 0.295	0%

Severe CSO PVS

(dichotomized around top quartile)

Same (+0%)	0.52,	0.39,	0.45,	0.45,	0.60,	0.69,	0.49,	0.47,	0.37,	0.59,	30%
	0.096	0.021	0.054	0.046	0.164	0.316	0.06	0.053	0.015	0.186	
+10%	0.62,	0.44,	0.50,	0.59,	0.35,	0.50,	0.38,	0.44,	0.62,	0.63,	40%
	0.187	0.028	0.064	0.150	0.007	0.068	0.019	0.033	0.17	0.185	
+20%	0.58,	0.45,	0.62,	0.47,	0.48,	0.47,	0.43,	0.47,	0.45,	0.36,	80%
	0.117	0.032	0.172	0.045	0.037	0.034	0.024	0.04	0.039	0.008	
+30%	0.80,	0.53,	0.73,	0.37,	0.58,	0.67,	0.69,	0.47,	0.61,	0.40,	20%
	0.502	0.058	0.332	0.008	0.113	0.231	0.282	0.051	0.150	0.013	

Model 2 - adjusted for age, sex, education, APOE-E4 carrier status, BMI, smoking status, hypertension, diabetes, WMH volume (log-transformed), presence of lacunes, presence of multiple (≥1) microbleeds.

Supplemental Table 4.10: Longitudinal relationship of basal ganglia (BG) and centrum semiovale (CSO) perivascular spaces (PVS) with Global Cognition over four years, for those patients without dementia at baseline (n=400); parameter estimate - unstandardized (SE) for the PVS*wave interaction term.

Cognition	Any severe PVS (Severe BG or severe CSO PVS)		Both severe PVS (Severe BG and severe CSO PVS)		Severe BG PVS (dichotomized around top quartile)		Severe CSO PVS (dichotomized around top quartile)	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
	B (SE), p-value	B (SE), p-value	B (SE), p-value	B (SE), p-value	B (SE), p-value	B (SE), p-value	B (SE), p-value	B (SE), p-value
Global Cognition [a]	-0.054 (0.042), 0.198	-0.054 (0.042), 0.199	**-0.139 (0.076), 0.045**	-0.140 (0.076), 0.065	-0.023 (0.049), 0.640	-0.022 (0.049), 0.642	**-0.104 (0.048), 0.029**	**-0.104 (0.048), 0.030**

Model 1 - adjusted for age, sex, education, APOE-E4 carrier status, BMI, smoking status, hypertension and diabetes.

Model 2 - adjusted for the covariates in Model 1 and WMH volume (log-transformed), presence of lacunes, presence of multiple (≥1) microbleeds.

Bold text indicates significance level of $p<0.05$.

Data are missing on two or three of the assessment waves for [a]23 participants.

Supplemental Table 4.11: Longitudinal relationship of basal ganglia (BG) and centrum semiovale (CSO) perivascular spaces (PVS) with individual cognitive tests over four years. (N=414); parameter estimate - unstandardized (SE) for the PVS*wave interaction term.

Cognitive test	Any severe PVS (Severe BG *or* severe CSO PVS)		Both severe PVS (Severe BG *and* severe CSO PVS)		Severe BG PVS (dichotomized around top quartile)		Severe CSO PVS (dichotomized around top quartile)	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
	B (SE), p-value	B (SE), p-value	B (SE), p-value	B (SE), p-value	B (SE), p-value	B (SE), p-value	B (SE), p-value	B (SE), p-value
Digit Symbol-Coding[297]	0.004 (0.034), 0.917	0.003 (0.034), 0.923	-0.092 (0.061), 0.195	-0.092 (0.061), 0.131	-0.025 (0.040), 0.535	-0.024 (0.040), 0.542	-0.009 (0.039), 0.856	-0.010 (0.039), 0.797
Trail Making Test A[298] (lg transformed)	0.012 (0.009), 0.186	0.012 (0.009), 0.190	0.030 (0.017), 0.181	0.030 (0.017), 0.071	0.016 (0.011), 0.138	0.015 (0.011), 0.147	0.012 (0.010), 0.224	0.012 (0.010), 0.229
Boston Naming Test – 30 items[301] (lg transformed)	0.004 (0.006), 0.546	0.004 (0.006), 0.563	0.008 (0.011), 0.455	0.008 (0.011), 0.458	0.009 (0.007), 0.214	0.009 (0.007), 0.230	0.000 (0.007), 0.972	0.000 (0.007), 0.968
Semantic Fluency (Animals)[298]	-0.050 (0.049), 0.305	-0.048 (0.049), 0.327	-0.234 (0.086), 0.007	-0.232 (0.086), 0.007	-0.071 (0.057), 0.212	-0.068 (0.057), 0.228	-0.091 (0.055), 0.103	-0.091 (0.055), 0.098
Trail Making Test B[298] (lg transformed)	0.012 (0.009), 0.182	0.011 (0.009), 0.191	0.021 (0.016), 0.187	0.022 (0.016), 0.177	0.016 (0.010), 0.130	0.015 (0.010), 0.135	0.009 (0.010), 0.365	0.009 (0.010), 0.362
Controlled Oral Word Association Test (FAS)[298]	-0.035 (0.035), 0.309	-0.035 (0.035), 0.312	-0.091 (0.062), 0.168	-0.091 (0.062), 0.151	-0.052 (0.041), 0.202	-0.051 (0.041), 0.207	-0.034 (0.040), 0.418	-0.034 (0.040), 0.386

Rey Auditory Verbal Learning Test[298] – sum of trials 1-5	-0.028 (0.045), 0.534	-0.028 (0.045), 0.543	-0.211 (0.082), 0.010	-0.210 (0.082), 0.010	-0.039 (0.053), 0.458	-0.039 (0.053), 0.468	-0.083 (0.052), 0.099	-0.083 (0.052), 0.117
Rey Auditory Verbal Learning Test[298] –trial 6	-0.030 (0.044), 0.493	-0.029 (0.044), 0.509	-0.122 (0.079), 0.118	-0.121 (0.079), 0.125	0.014 (0.051), 0.784	0.016 (0.051), 0.755	-0.100 (0.050), 0.043	-0.100 (0.050), 0.043
Rey Auditory Verbal Learning Test[298] –trial 7	-0.001 (0.043), 0.984	0.000 (0.043), 0.999	-0.160 (0.077), 0.038	-0.159 (0.077), 0.040	-0.020 (0.050), 0.698	-0.018 (0.050), 0.722	-0.046 (0.049), 0.344	-0.047 (0.049), 0.345
Logical Memory[299]	-0.049 (0.048), 0.307	-0.048 (0.048), 0.320	-0.174 (0.086), 0.044	-0.173 (0.086), 0.046	-0.050 (0.057), 0.375	-0.049 (0.057), 0.390	-0.087 (0.055), 0.098	-0.086 (0.055), 0.118
Benton Visual Retention Test[300]	0.083 (0.062), 0.165	0.083 (0.062), 0.177	0.179 (0.113), 0.574	0.179 (0.113), 0.114	0.134 (0.072), 0.064	0.134 (0.072), 0.064	0.051 (0.070), 0.471	0.051 (0.070), 0.471
Block Design[302]	-0.039 (0.045), 0.387	-0.039 (0.045), 0.387	-0.103 (0.080), 0.106	-0.103 (0.080), 0.196	0.000 (0.052), 0.998	0.000 (0.052), 0.997	-0.092 (0.050), 0.068	-0.092 (0.050), 0.069

Model 1 - adjusted for age, sex, education, APOE-E4 carrier status, BMI, smoking status, hypertension and diabetes.

Model 2 - adjusted for the covariates in Model 1 and WMH volume (log-transformed), presence of lacunes, presence of multiple (≥1) microbleeds.

4.3 Summary of main findings

I found that participants with severe PVS pathology in both regions or in the CSO alone had greater decline in global cognition over four years, even after adjustment for the presence of other small vessel disease neuroimaging markers. There was no association between severe PVS in any of the four groups and decline in any particular cognitive domain however.

The presence of severe PVS pathology in both regions was an independent predictor of dementia across eight years (OR 2.91, 95%CI 1.43–5.95, p= 0.003). Further, the presence of severe PVS pathology in all groups examined was associated with greater dementia risk at either year four or six. This effect was not seen at year 8 however, suggesting that severe PVS pathology may be an early marker for incident dementia. This may be because those with severe PVS pathology may have dropped out earlier and the signal from PVS was lost due to the stronger effect of age over time.

The effect of PVS may differ by region, similar to the effect seen with CMB discussed in Chapter 2. Enlargement of PVS in the white matter is reportedly associated with cerebral amyloid-β pathologies and CSO PVS pathology might indicate the presence of CAA or a mixed hypertensive / CAA[4,35-37] pathophysiology. In contrast, BG PVS are a marker for hypertensive arteriopathy.

The strongest findings were for those 32 (7%) participants with severe PVS in both regions. This could be due to two non-exclusive mechanisms. First, that this group has the most severe and widespread PVS pathology. Alternatively, or additionally, there may be an additive effect of the two different pathologies present in the different regions – hypertensive arteriopathy and CAA, which together produce a larger association with cognitive decline and dementia.

This study confirmed that PVS should not be considered an incidental finding, but are useful when assessing the impact of CVD and may be able to aid diagnosis and prognosis of cognitive impairment and dementia. Their presence should be assessed in the constitution of any multi-modal CVD index and consideration given to separate assessment of regional pathology.

Chapter 5: Development and validation of a novel MRI index to quantify the relationship of cerebrovascular disease with cognition.

Abstract

Objective:

To develop an MRI-based composite cerebrovascular disease (CVD) index and validate it in two independent cohorts. Further, to compare its performance to an established CVD index as well as two component neuroimaging markers – white matter hyperintensity (WMH) volume and Peak Skeletonised Mean Diffusivity (PSMD).

Methods:

424 participants in the Sydney Memory Ageing Study (MAS) underwent multi-modal MRI and had a full neurocognitive assessment. Through univariate analysis, four individual MRI makers were selected for consideration of entry into the index: whole brain WMH volume; the presence of cerebral microbleeds, the presence of lacunes and PSMD. The index was computed based on the result of the multiple regression linear analysis in predicting Global Cognition scores. This index was then validated in two independent cohorts, the Older Australian Twin Study (OATS) and the Renji Cerebral Small Vessel Disease Cohort Study.

Results:

Of the four component MRI markers, PSMD was the largest contributor to the index, with a standardized regression coefficient (β) of -0.36, followed by WMH volume (β of 0.11) and microbleeds and lacunes (β of -0.03) each. The index explained 9% of the proportion of variance (R^2) of Global Cognition in the MAS and when applied to the validation cohorts, had an R^2 of 0.05 in the OATS and 0.13 in the Renji. In all cohorts examined, the index explained more of the variation in Global Cognition than the Staals CVD index.

Conclusions:

The novel CVD index included both structural and diffusion tensor imaging measures of small vessel disease. It outperformed an existing CVD index, but had variable results when validated on heterogenous datasets, highlighting the challenge of utilizing a single index across a range of cohorts. The index represents a promising step-forward in the assessment of CVD, which could potentially help clinicians and researchers in determining the CVD burden that may underlie dementia. Further work is needed on refinement of the index, including possible future inclusion of other non-structural imaging modalities.

Introduction

Cerebrovascular disease (CVD) disease is very common - post-mortem examination shows 50% to 84% of the brains of people who die aged 80 or older have appreciable cerebrovascular lesions[4].

Its presentation is pleomorphic, with multiple different lesions seen as a consequence of damage to blood vessels and surrounding parenchyma. These may represent several distinct, but possibly overlapping pathologies. Broadly, CVD has been divided into large vessel disease (LVD) and small vessel disease (SVD). LVD is commonly due to atherothrombotic occlusion of cerebral arteries. SVD can result from multiple pathologies; most commonly arteriolosclerosis but other causes include sporadic and hereditary cerebral amyloid angiopathy and genetic small vessel diseases – see Table 1.1.

The clinical manifestations of these lesions are also very heterogeneous. LVD can lead to ischaemic or haemorrhagic strokes as well as certain types of vascular cognitive disorders[1]. SVD can have manifold clinical consequence, including lacunar stroke, vascular cognitive disorders, gait disturbance, urinary incontinence and neuropsychiatric symptoms. The contribution of SVD to the risk and progression of AD is also being increasingly recognized[9].

Many of these lesions can be visualized directly or indirectly with MRI, which has become the most commonly used brain imaging modality to estimate CVD. There is considerable variability in presentation however and there are individuals with substantial MRI-defined CVD who do not have significant cognitive or functional sequalae of the presumed pathology. Equally, there are individuals with significant deficits, with little visualized pathology.

In a clinical setting, for an accurate dementia diagnosis, a clinician visualizes the different lesions and estimates the overall burden of CVD for a given patient. However, there is little guidance for the clinician on how the different lesions must be combined and how the various elements could be weighted. There have been efforts to solve this problem, including a widely used scale[107], which equally weights four common SVD markers, but this approach may be overly simplistic and lacks non-structural approaches. Other attempts[107, 108, 181, 182] have been limited by lack of

weighting of variables, limited number of constituent lesions, lack of consideration of LVD and exclusion of functional imaging parameters.

The ideal approach would be to relate MRI findings to neuropathology, but this has major limitations, because, as discussed in a recent review[2], there is no consensus agreement on the quantification of CVD neuropathology. There is also limited data examining antemortem MRI close to time of death.

In the absence of neuropathology, a practical solution is to use the relationship between MRI markers and cognition as a functional proxy for the impact of CVD. Cognitive measures are reliable, easily available and obtained concomitantly with the neuroimaging.

Our primary aim was to develop a novel MRI CVD index, maximally associated with cognition, and to test its properties in two separate validation cohorts. Our secondary aim was to test its performance with the i) best established CVD index[107], ii) WMH volume as the traditional marker of SVD and iii), PSMD[51], the individual marker best associated with cognition. Our hypothesis was that an index will perform better than the best individual markers of CVD.

Methods

I developed the Index from the Sydney Memory and Ageing study (MAS) and tested its performance on two validation cohorts – the Older Adult Twin Study (OATS) and the Renji Cerebral Small Vessel Disease Cohort Study (RCCS).

Development cohort: Sydney Memory and Ageing study (MAS)

The MAS[151] is a population based longitudinal study of older adults, designed to examine predictors of cognitive decline in a non-demented, community-dwelling sample. Participants were aged 70 to 90 at study inception and their baseline assessment included a medical examination, comprehensive neuropsychological assessment and MRI scan. They were then followed up every two years. For this study, I used data from Wave 2, when a more comprehensive MRI examination was introduced. There were 424 participants in this cohort with sufficient MRI data and cognitive data for analysis.

Radiological examination:

All MRI scans were performed on a Philips 3T Achieva Quasar Dual scanner (Philips Medical Systems, Best, Netherlands). A 3D T1-weighted sequence (1×1×1 mm3, TR/TE=6.39/2.9 ms), a T2-weighted fluid attenuation inversion recovery (FLAIR) sequence (TR/TE/TI=10000/110/2800 ms; thickness 3.5 mm; 0.898×0.898 mm2), diffusion weighted imaging (DWI) (b1=1000s/mm^2, 32 non-collinear directions, 2.5 mm^3 isotropic voxels) and a susceptibility weighted imaging sequence (parameters previously described[71]) were performed. T2-FLAIR and SWI images were co-registered to T1 images using Statistical Parametric Mapping version 12[370] (SPM 12). All images were analyzed using MRIcron version 15 (www.nitrc.org/projects/mricron/).

The following MRI markers of CVD were then quantified:

- Total WMH volume: assessed with automated methods using FLAIR and T1-weighted images[148], and adjusted for total intra-cranial volume[40]. Separate volumes for periventricular (PV) and deep white (DW) matter WMH were also produced.

- WMH severity (visual rating): this was assessed using the Fazekas visual scale[147], which included separate scores for periventricular and deep white matter severity. Rating was done by an experienced psychiatrist (MP), based on a training set[371].

- Perivascular spaces (PVS): the number of PVS were counted in a single axial slice in the BG and centrum CSO respectively according to a recently published scale[372]. In brief, PVS were counted on two pre-defined T1-weighted axial slices, 2mm and 37mm superior to the anterior commissure. PVS were defined according to STRIVE[38] criteria, i.e. they have CSF signal intensity and follow the course of penetrating vessels, appearing linear when viewed parallel to the course of a penetrating vessels or round or ovoid when imaged perpendicular to the vessel. They were distinguished from lacunes by the lack of a hyperintense rim on FLAIR sequences and by size. In contrast to PVS, lacunes are usually greater than 3mm in diameter but I included PVS of any diameter if they fulfilled other diagnostic criteria.

- Lacunes: were defined using STRIVE criteria[38] and the total number counted.

- Cerebral microbleeds (CMB): CMB were defined as round hypointense foci <10 mm in diameter seen on SWI, at least half surrounded by brain parenchyma and distinct from potential mimics such as iron and calcium deposits, vessel flow voids and bone[68]. Symmetrical hypointensities in the globus pallidus likely representing calcium or non-hemorrhagic iron deposition were excluded. Hypointensities within the subarachnoid space were deemed to be pial blood vessels. The co-registered T1 images were used to determine precise anatomical localization and help exclude sulcal flow voids. CMB were rated and localized using a published scale (MARS[309]), with the numbers in the infratentorial, deep and lobar regions counted.

- Large vessel disease (LVD): this was defined as the presence of any cortical infarct, recorded during the initial neuroradiologist's reporting of the MRI scan. The total number of infarcts were counted.

- Peak Skeletonised Mean Diffusivity (PSMD[51]): This is a novel diffusor tensor imaging (DTI) metric, defined as the defined as the difference between the 95th and 5th percentiles of skeletonized mean diffusivity. DWI data were

first skeletonized using Tract-Based Spatial Statistics (TBSS), part of the FMRIB Software Library[373]. Mean diffusivity (MD) images were projected onto the resulting skeleton using fractional anisotropy (FA) derived projection parameters. These images were further masked using a standard skeleton threshold at an FA of 0.3 and a mask provided with the PSMD tool to exclude regions adjacent to the ventricles [51]. Mean MD values were calculated by averaging each individual's skeletonized MD data and a PSMD value generated.

For later comparison, a Staals index score[107] was generated, which rates lesions on a 0-4 scale. It is the sum of the presence of ≥1 lacune, a periventricular Fazekas score of 3 or a deep white Fazekas score of 2-3, ≥1 microbleed and moderate to severe PVS pathology in the basal ganglia, defined as the upper quartile of severity. For the MAS and OATS cohorts, as per the original scoring[183], just the presence of deep CMB were included. For the Renji, where CMB regional data was not available, CMB anywhere in the brain were utilized.

Covariates:

Hypertension was defined as a blood pressure of ≥140/90 mm/Hg taken as the mean of two seated readings or if the participant was ever diagnosed as having hypertension by their doctor. Diabetic status was determined by a prior medical diagnosis or a fasting blood glucose value ≥7 mmol/L.

Neuropsychological assessment:

Research psychologists administered a battery of tests, grouped into cognitive domains: attention and processing speed (Digit Symbol-Coding[297], Trail Making Test A[298]), executive function (Controlled Oral Word Association Test[298], Trail Making Test B[298]), language (Boston Naming Test – 30 items[301], Semantic Fluency (Animals)[298]), visuospatial (Block Design[302]), and memory (Logical Memory[299], Rey Auditory Verbal Learning Test[298], Benton Visual Retention Test[300]).

At each study wave, raw test scores were transformed to z-scores using the Wave 1 mean and SD values of a healthy reference group, n=723 MAS participants. Domain scores were calculated by averaging the z-scores of component tests. These

composite scores were then standardized as z-scores, calculated using the means and SDs for the Wave 1 healthy reference sample. A global cognition score was calculated by averaging all domain scores and again, transforming this to a z-scores using the Wave 1 healthy reference sample.

Validation cohort: Older Australian Twin Study (OATS)

The OATS[374, 375] is a longitudinal study of twins aged 65 and over which began in 2006. The initial cohort comprised 623 individuals, of whom 404 had an MRI scan, over three states: Sydney, Melbourne and Queensland.

A comprehensive neuropsychological assessment was performed at baseline with both a computerized battery and paper and pencil testing. Tests were grouped into the cognitive domains of attention and processing speed, executive function, language, visuospatial ability, and memory (see details[17]). Domain scores were formed from a composite comprising the average of the Z-scores of the tests included in each domain, using the means and standard deviations at Wave 1. These were then standardized and a Global cognition score obtained using the same method as in the MAS.

Radiological examination:

MRI was performed in three centers in Wave 1: Sydney, Melbourne and Brisbane. Matching acquisition protocols were used in the three centres, with standardization of in-plane resolution and slice thickness. The following protocol was used for T1-weighted MRI scans on the 1.5 T scanners in all three centres: in-plane resolution 1×1 mm with slice thickness of 1.5 mm, contiguous slices (TR/TE/TI = 1530/3.24/780 ms). FLAIR scans were acquired axially with the same acquisition parameters on the 1.5 T scanners in all three centres (TR/TE/TI = 10,000/ 120/2800 ms, with slice thickness 3.5 mm and in-plane resolution 0.898×0.898 mm2). On the 3T scanner in Sydney, there was a spatial resolution of $1 \times 1 \times 1$ mm3, TR/TE = 6.39/2.9 ms for T1-weighted scans, and TR/TE/TI = 10,000/110/2800 ms, with slice thickness 3.5mm and in-plane resolution 0.898×0.898 mm2 for FLAIR scans. DWI (b1=1000s/mm^2, 32 non-collinear directions, 2.5 mm^3 isotropic voxels) and SWI sequence was performed in one center (Melbourne) - parameters previously described[374, 375]. Image analysis

and quantification of CVD lesions followed the same protocol as described in the MAS above.

Validation cohort: Renji Cerebral Small Vessel Disease Cohort Study (RCCS).

The RCCS[245] is a cohort study of post-stroke cerebral SVD patients with differing levels of cognition, but no dementia (Renji Cerebral SVD Cohort Study, RCCS, http://www.clinicaltrials.gov, NCT01334749). 127 participants were recruited consecutively from the stroke clinic at Renji Hospital, Shanghai Jiao Tong University School of Medicine from 2015 to 2018. 43 age and sex matched healthy community-dwelling participants without CSVD were recruited from the Tangqiao community, Pudong New District in Shanghai as normal controls (NC). Detailed recruitment and exclusion criteria for CSVD patients have been published elsewhere[245].

Each subject underwent a standard baseline evaluation including neurological examination, complete sociodemographic and clinical data, neurologic examination, neuropsychological assessment and multimodal MRI examination. Participants were aged 50 to 85 years, seen at least one month after the clinical lacunar stroke, demonstrated the presence of subcortical lacunar infarct(s) and WMH on MRI and had a modified Rankin score ≤ 3 points[376].

A detailed battery of cognitive tests was administered by trained neuropsychologist, allowing grouping into the following cognitive domains; processing speed, executive function, language, visuospatial function and memory. Domain scores were calculated by averaging the corresponding tests and standardizing using the means and SDs of the healthy reference sample. Global cognition scores were computed in a similar way by averaging the domain scores and subsequently transforming to z-scores.

MRI acquisition. Standardized T1-weighted 3D fast spoiled gradient recalled (SPGR) sequence images, T2-weighted FLAIR, and DWI were obtained using a 3T MRI scanner (Signa HDxt; GE HealthCare, Milwaukee, WI, USA). Detailed parameters can be found in a previous publication[245].

Standard Protocol Approvals, Registrations, and Patient Consents:

Written informed consent was obtained from all participants. Ethical approval was received from the University of New South Wales Human Research Ethics

Committee[151]. The reporting of this study is compliant with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines[358].

Data Availability Statement:

Anonymized data from the MAS, OATS and RCCS are available on request.

Analysis:

Outcome variable:

Global cognition was used as the outcome in the analysis. Although vascular cognitive disorders are more commonly associated with impairments in executive function and attention and processing speed[1, 23], due to the variability in presentation all cognitive domain can be affected and Global Cognition was chosen as a proxy for the functional impact of CVD. As a sensitivity analysis, I explored through the use of a canonical correlation whether a weighted composite of cognitive measures would produce a stronger association with the MRI measures. Methods and results are presented in Supplement Table 5.4.

MRI marker selection:

To determine which MRI measures were to be included in the Index and their scaling, univariate regression analysis of associations with Global Cognition were performed. For the continuous variables of WMH volume and PSMD, quadratic terms were used to see if this strengthened the association with the DV. Interactions terms were also tested between the MRI measures, after centering of continuous variables.

Index development:

Multivariate linear regression was performed on the MAS cohort to examine the predictive power of the index on Global Cognition in the development cohort. Each MRI marker which had at least a borderline significance ($p < 0.1$) with Global Cognition on univariate analysis was entered simultaneously in the model. The proportion of variance (R^2) explained by the Index was used to indicate model fit.

Multiple imputation by chained equations was performed using the mice package in R to handle missing data[377]. Only cases with two or more MRI measures were included in the imputation. Demographic and health variables of age, sex, education,

diabetes, hypertension, smoker, and BMI were used as auxiliary variables in the imputation. One thousand imputations were chosen to estimate the parameters by applying Rubin's rules to pool across imputations, and to construct confidence intervals of these pooled estimates.

Index validity:

The index developed in MAS was then tested in the OATS and Renji cohorts. A multivariate linear regression using the regression intercept and coefficients generated in MAS was applied to the OATS data to compute predicted values of Global Cognition, which were then compared against the observed values by computing model R^2. Again, multiple imputation was performed with pooled estimates and confidence intervals computed across 1,000 imputations. The same procedure was applied to the Renji cohort.

The confidence intervals of adjusted R^2 from the multiple MRI markers model was compared to those produced by i) Staals CVD index[107], ii) WMH volume, and iii), PSMD[51]. Potential overlap in the 95% CI indicates there is no significant difference in variance explained in the models compared.

Sensitivity analyses:

Given a large number of OATS participants did not have SWI (n=143; 34%) and were therefore missing CMB data and that the Renji cohort did not contain detailed information about CMB, a second set of model was created, excluding CMB. Following this, similar analysis detailed above was performed.

An additional analysis was trialed, pooling the MAS and OATS together to create a new development cohort. The resultant index was then validated on the Renji cohort.

All analyses were performed using R Version 4[378] and the SPSS statistical package (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

Results

Cohort characteristics:

Table 5.1 reports demographic information on the three cohorts utilized. There were 424, 415 and 171 participants in the MAS, OATS and Renji cohorts respectively. There are differences between the groups, with the average age of participants ranging from 65.4 [SD 7.7] in the Renji cohort, to nearly 80 in the MAS (mean 79.8 [SD 46]. There are also large differences in the proportion of male participants (76% of the Renji cf. 47% of the MAS). As a pathological cohort of post stroke patients, the Renji also has a higher degree of CVD neuroimaging pathology, particularly when compared to the population based younger OATS study. There are a greater number of participants with lacunes, microbleeds and severe basal ganglia PVS. The Renji participants have on average, greater WMH volume and PSMD.

Table 5.1: Characteristics of the study cohorts.

Variable	Sydney Memory and Ageing Study (MAS)		Older Australian Twin Study (OATS)		Renji Cerebral Small Vessel Disease Cohort Study (RCCS).	
	Total N (%) or Mean ± SD	N missing	Total N (%) or Mean ± SD	N missing	Total N (%) or Mean ± SD	N missing
Total	**424**		**415**		**171**	
Demographics						
Age, years	79.8 ± 4.6	1	70.9 ± 5.1	1	65.4 ± 7.7	0
Men	199 (47)	1	143 (35)	1	130 (76)	0
Education, years	12.0 ± 3.7	1	11.4 ± 3.5	1	10.6 ± 3.0	3
MMSE	28.2 ± 1.7	1	28.4 ± 1.9	6	29.0 ± 19.7	10
Vascular risk factors						
BMI	26.9 ± 4.0	11	27.3 ± 4.3	50	N/A	-
Smoker (in last month)	19 (5)	43	20(5)	6	76 (50)[a]	19
Hypertension	338 (80)	3	244 (59)	1	118 (74)	15
Diabetes	83 (20)	2	43 (10)	1	58 (37)	14
APOE-ε4 carrier	98 (23)	3	107 (28)	28	N/A	-
Neuroimaging markers						
WMH volume cm^3	22.00 ± 18.34	11	7.63 ± 10.84	3	25.84 ± 46.78	0
Presence of lacunes	35 (8)	4	14 (3)	2	108 (93)	55
Presence of cerebral microbleeds	81 (22)	57	26 (6)	272	49 (42)	55
Presence of severe BG PVS	90 (22)	10	71 (17)	4	33 (28)	55
Presence of severe CSO PVS	99 (23)	10	101 (24)	4	33 (28)	55

Presence of cortical infarct	24 (6)	10	N/A	-	N/A	-
PSMD *10^{-4} mm^2/s	4.15 ± 0.77	4	3.14 ± 0.70	21	5.30 ± 1.26	0

[a] The Renji cohort operationalized smoking status as current or previous vs. never smoked.

Abbreviations: SD, Standard Deviation; MMSE, Mini Mental State Examination; BMI, Body Mass Index; WMH, White Matter Hyperintensity; CMB, cerebral microbleeds; PVS, Perivascular spaces; PSMD, Peak Skeletonised Mean Diffusivity.

Index construction:

Outcome variable:

The canonical correlation R^2 using all cognitive domains and MRI measures was 0.159, with no single domain exerting a clear dominance. The equivalent R^2 from a regression analysis using all MRI measures and Global Cognition was 0.130. I was therefore justified in using Global Cognition as the outcome measure.

MRI measures selection:

Supplemental Table 5.5 details the results of the exploratory univariate analysis for different scaling of the putative MRI measures.

The following measures and their scaling were included in the development of the Index.

1) WMH volume across the whole brain, as a continuous, automated measure.
2) CMB, operationalized as a binary variable, presence or absence anywhere in the brain.
3) Lacunes, defined as the presence of any lacunes anywhere in brain. This was a binary variable, present vs absent.
4) PSMD, as a continuous, automated measure.

25 MAS participants (6%) had imaging evidence of previous cortical infarcts. The presence of LVD was not associated with global cognition on univariate analysis; (mean (SD) Global cognition of participants with LVD; -0.40 (1.09); mean (SD) Global cognition of participants without LVD; -0.14 (1.18); t = 1.04, p = 0.30). Nor were there any interaction terms with other MRI measures or associations in multivariable analysis. Being present in just 6% of participants, it was not appropriate to subdivide LVD or scale it other than dichotomously. Large vessel disease was therefore not included in the Index.

Perivascular space severity was not also included in the Index and there were no univariate associations with global cognition. This was the case when examining the number of PVS across the whole brain, the number in the BG and the number in the CSO as continuous measures as well as dichotomizing PVS into only those with

severe (top quartile) PVS pathology in the BG or CSO regions. There were no interaction terms with other MRI measures or associations in multivariable analysis.

For cerebral microbleeds, individuals with deep microbleeds had the strongest associations with impaired global cognition. Given there were only 17 participants (5%) with deep CMB and the Renji cohort did not rate regional CMB, the decision was made to include CMB anywhere in the brain, despite the weaker and borderline association with global cognition (B = -0.25, p =0.101).

Quadratic terms for WMH volume and PSMD did not improve the association and were not used. There were no significant interaction terms between the final four measures included in the index.

Missing data:

This is detailed in Table 5.1: Cohort characteristics. There was a noticeable amount of missing information on CMB. 57 (13%) of the MAS participants did not have this data and nor did 272 (66%) of the OATS participants, as SWI imaging was only performed in one of the three OATS centers. In the Renji, visually rated information on lacunes, CMB and PVS was missing in 55 (32%) participants.

CVD index:

Table 5.2 shows the standardized and unstandardized coefficients for the multiple linear regression, derived from the development sample (MAS). PSMD was the strongest predictor (β = -0.36, p <0.001), followed by WMH volume (β = 0.11, p =0.12). In the presence of PSMD and WMH, the presence of lacunes and microbleeds added little to the model (β = -0.03, p =0.59 and β = -0.03, p =0.63, respectively).

Table 5.2: CVD Index – multiple regression result for Global Cognition (Sydney Memory and Ageing Study, imputed data, N=421).

CVD Index	B	95.0% CI for B		SE B	β	R^2	Adj. R^2
		LL	UL				
Model						0.09	0.08
Constant	2.15	1.32	2.99	0.42			
Whole brain WMH volume (cm3)	0.01	-0.00	0.02	0.00	0.11		
Presence or absence of cerebral microbleeds	-0.07	-0.37	0.22	0.15	-0.03		
Presence or absence of lacunes	-0.11	-0.52	0.30	0.21	-0.03		
PSMD $*10^{-4}$ mm^2/s	-0.55	-0.75	-0.34	0.10	-0.36***		

B = unstandardized regression coefficient; CI = confidence internal; LL = lower limit; UL = upper limit; SE B = standard error of the coefficient; β = standardised coefficient; R2 = coefficient of determination; Adj. R^2 = adjusted R^2

* $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$

Table 5.3: CVD Index model summary: development, validation and comparison with other measures. Confidence intervals were computed based on 1000 imputed datasets. Adjusted R^2 values presented.

Table 5.3a: CVD index development on MAS and validated on OATS and Renji cohorts: associations with Global Cognition.

Method	Measures in model	Development cohort (MAS) (N=421)		Validation cohort (OATS) (N=413)		Validation cohort (Renji) (N=171)	
		R^2	95% Lower and upper bound	R^2	95% Lower and upper bound	R^2	95% Lower and upper bound
CVD Index value, **developed on MAS***	PSMD, WMH volume, Lacunes, CMB	0.085	(0.040 - 0.143)	0.048	(0.028 - 0.072)	0.128	(0.067 - 0.199)
Alternative CVD Index, without CMB, **developed on MAS***	PSMD, WMH volume, Lacunes,	0.083	(0.039 - 0.141)	0.053	(0.033 - 0.078)	0.138	(0.076 - 0.209)

Table 5.3b: Association of MRI measures with Global Cognition in each cohort.

Method	MAS (N=421)		OATS (N=413)		Renji (N=171)	
	R^2	95% Lower and upper bound	R^2	95% Lower and upper bound	R^2	95% Lower and upper bound
Staals index	0.007	(0.000 - 0.033)	0.038	(0.005 - 0.095)	0.059	(0.003 - 0.172)
PSMD	0.082	(0.038 - 0.139)	0.068	(0.027 - 0.123)	0.251	(0.138 - 0.375)
WMH volume	0.020	(0.002 - 0.057)	0.057	(0.021 - 0.107)	0.135	(0.042 - 0.260)

Table 5.3c: CVD index development on combined MAS-OATS cohort and validated on the Renji cohort: associations with Global Cognition.

Method	Measures in model	Development cohort (MAS and OATS) N=834		Validation cohort (Renji) N=171	
		R^2	95% Lower and upper bound	R^2	95% Lower and upper bound
CVD Index value	PSMD, WMH volume, Lacunes, CMB.	0.089	(0.054 - 0.130)	0.235	(0.149 - 0.326)

Staals scale: presence of lacunes, presence of deep CMB, moderate to severe dPVS in basal ganglia and extensive WMH (periventricular severity 3 or deep WMH severity 2–3).

For the main analysis, (Table 5.3a) the CVD index had an R^2 of 0.09, but the proportion of variance drops considerably when the regression equation above is applied to the OATS cohort, with an R^2 of just 0.05. In contrast, in the Renji cohort, the applied CVD index score explains a greater amount of variance: with an R^2 of 0.13.

The Staals explained less than 1% of the proportion of variance in MAS (Table 5.3b), a significant worse performance than the CVD index as estimated by non-overlapping 95% R^2 range. This did not replicate in the validation cohorts, and although the Staals index had a lower R^2, the confidence intervals of the Staals and the CVD index overlapped. WMH had a lower R^2 in the development cohort (0.02), but similar values to the CVD index in the validation cohorts. PSMD explained a large amount of variance in all cohorts examined, with an R^2 of 0.25 in the Renji cohort but again, the confidence intervals of PSMD overlapped with the CVD index.

Secondary analysis:

When an alternate index was developed excluding CMB (regression model reported in Supplemental table 5.6), the performance was similar to the original full index, with negligible differences in R^2 in both the development and validation cohorts.

When the index was developed on a pooled cohort of MAS and OATS (Table 5.3c), the proportion of variance explained in the Renji validation cohort was 0.24 compared to 0.13 in the original cohort.

Discussion

I developed and validated a novel CVD index, to better capture the variability of cerebrovascular burden – represented as the association with global cognition. I found that the index performed well in the development cohort, but performance was variable when validating on separate cohorts, with R^2 values ranging from 5 to 13%. This performance is likely due to the differing characteristics of the cohort. The OATS is a younger, healthier community cohort than the MAS, with less CVD pathology. There is also less variance and impairment in global cognition and overall the MRI index explained less of global cognition. In contrast, the Renji is a cohort of post-stroke patients, who have relatively large degrees of CVD pathology and greater amounts of cognitive impairment. Here, the index performed very well, with a greater R^2 than the development cohort.

When compared to existing methods of CVD estimation, in the development cohort, our index explained a significantly greater proportion of variance than the best-known CVD index, the Staals[107]. However, when applied to the validation cohorts, the index did not demonstrate a statistically significant benefit compared to the measures of WMH volume and PSMD.

Indeed, on both validation cohorts, the R^2 of PSMD appeared larger than the R^2 of the CVD Index. When multiple regression analyses were performed for each validation cohort separately (Supplemental Table 5.8), in both these cohorts, the overall proportion of variance explained by all MRI measures was greater than PSMD. I.e., the models were significantly improved by the inclusion of the other non-PSMD MRI measures. This suggests that the thesis that a multi-modal index is more informative than a single measure is valid, but the challenge is the applicability of an index developed in a different cohort. Specifically, applying the weightings derived from the MAS on heterogeneous validation cohorts with differing characteristics. The multiple regression analyses in Supplemental Table 5.8, demonstrate that different coefficients were produced for the same MRI measures in each of the cohorts. WMH volume for example, was significantly associated with global cognition in OATS but not the Renji cohort.

There is significant overlap between the MRI measures in the index, which are thought to represent predominantly SVD pathology. PSMD is associated with WMH volume, lacunes and microbleeds[379] and the markers may also signify different points on a time continuum, i.e., the early damage revealed by PSMD becoming WMH, which may evolve into lacunes[38]. There is not total collinearity between MRI measures however and their differential associations with cognition suggest each may be contributing unique information.

There were no regional measures (either CMB or PVS) included in the final model. Neither of these markers assessed globally contributed much to the proportion of variance; PVS was not included in the final regression and in the alternative CVD index where CMB was not included, the performance did not significantly suffer. (Supplemental table 5.6). The potential signal from *regional* differences in these markers was too weak to be included in the Index. Large vessel disease was also not associated with impairment in global cognition, but the MAS was a relatively healthy population cohort, and only 6% of participants had a cortical infarct, of unknown age. There may have also been a self-selecting bias, with only those participants relatively unaffected by previous infarct being able to participate in the study. The study was probably underpowered to detect an effect from LVD.

In the MAS multiple regression analysis, PSMD was the most powerful predictor, explaining the largest proportion of variance in global cognition. This supports the original PSMD development paper, where PSMD outperformed WMH volume, lacune volume and microbleed count in a range of cohorts including CADASIL, sporadic SVD and a memory clinic[51], albeit in association with processing speed. The superior association of DTI with cognition over structural MRI measures has been widely reported[51, 171, 282, 380]. DTI is a sensitive measure to early pathology, showing damage to white matter microstructure across the brain even in normal-appearing white matter on structural imaging. The histogram analysis used in PSMD is a sensitive method to quantify diffuse pathological changes, capturing the distribution of diffusivity values across the whole brain.

I found that a larger pooled cohort, of both the MAS and OATS produced an Index score that performed better in the Renji validation cohort, compared to the original MAS-derived CVD index. This could be due to a more heterogeneous sample and the increased size of the combined sample leading to more accurate estimates in the regression. Further study is required.

Relation to existing literature:

A 2018 systematic review found [2] four CVD indices, not including manuscripts where these indices were developed. Since then, there have been an addition seven indices, summarized in Supplemental Table 5.7. The majority have used come combination of the common SVD measures- WMH, lacunes, CMB and PVS. Dickie et al.[222] developed an overall Brain Health Index, with a novel automated approach, using several MRI sequences to classify voxels into healthy or pathological tissue. This index is not specific to CVD but did correlate with an existing SVD scale[107]. Three other scales[223-225] included a measure of atrophy, which can be a feature of CVD[38, 107], but is also seen in a variety of pathological and physiological process, including neurodegenerative disease and normal ageing.

Although direct comparison with indices other than Staals[107] were not performed, I would argue that our index has several advantages. First, it includes a DTI measure, PSMD. The results suggest that DTI has the strongest association with cognition, both in univariate and multivariate analysis. PSMD betters correlates with cognition that MD and FA, replicating the original development paper[51]. Second, where possible, the index relies on automated volumetric assessment, rather than visual rating. Univariate analysis showed that WMH volume as a continuous measure is more informative than using the Fazekas scale, as it is more reliable and has greater granularity in measurement. Third, the CVD index was developed empirically, with a variety of measures and their scaling considered.

Limitations:

There were several limitations of the study. First, there were missing data, particularly with the ratings of CMB. 24% of the MAS and 66% of the OATS (as the SWI protocol was only introduced in one centre) was missing CMB data and the Renji only detailed

global but not regional CMB information. Other demographic, and health data was missing, and I attempted to the mitigate the impact of this through multiple imputation methods.

Second, the cohorts are relatively small and very heterogenous. This limited the proportion of variance explained in the validation cohorts. However, heterogeneity of cohorts is needed to demonstrate ecological validity of the index, i.e., that it will perform well in a variety of patient populations, severities of cognitive impairment and different comorbidities. The limited number of participants does restrict the use of finer region-based lesion analysis[149], as discussed below.

There are also limitations to the widespread use of the index. The index relies on technology not necessarily available to all clinicians or researchers, including software necessary to automate WMH volume and to calculate PSMD. Also, to produce DTI data, a minimum number of diffusion-encoding gradient directions are required, which is not always standard in a clinical dementia protocol. An index score may also be difficult to interpret for a single patient although if a score is generated for a whole cohort, then it can be standardized and contextualized across the individuals in that group.

Future directions:

Given the difficulty of contextualizing an individual score, I plan to automate the index as an online calculator. Index scores can then be compared to age and gender matched participants in the development cohort. An automated mechanism could also save the user time, compared to manual calculation.

I plan to validate the index against other phenotypes of interest, including longitudinal cognitive change and incident dementia.

I would hope the index can be updated to incorporate potential modifications on three levels. One, changes to individual MRI measures. Although I did not find inclusion of regional markers helpful, further exploration of regional based approaches[149] should also be explored. Based on mapping white matter lesions onto specific tract for example, a recent paper has shown that regional white matter lesions are associated

with impairments in cognition, independent of the total white matter lesion load[47]. Two, incorporation of new, non-structural MRI modalities. This could include perfusion imaging such as including arterial spin labeling[381] (ASL), which does not rely on an exogenous tracer. ASL can provide perfusion information on a global or localized scale and changes in regional brain function may be more dynamic and show greater sensitivity to early disease, disease progression, or responses to therapy than changes in traditional structural MRI volumetric [381, 382]. Three, expansion of the number and range of included cohorts. This should ideally provide enough power to investigate multiple regions of interest and rarer lesions, providing a more representative cohort. Multiple cohorts would also allow validation in a range of populations.

In conclusion, I developed an MRI index that more accurately estimated CVD burden, represented by global cognition, that traditional methods. The inclusion of a DTI measure was powerful and although much more work is needed to refine the index, it represents a valuable tool in the diagnosis and monitoring of individuals with cognitive impairment and dementia.

Supplemental data

Supplemental Table 5.4: Canonical correlation coefficient results.

Canonical correlation was performed with all MRI measures and all cognitive domains included. Canonical correlation is a procedure designed to allow for the estimation of correlation coefficients between sets of variables. An equivalent multiple regression was then performed, using with all MRI measures and one dependent variable, the Global Cognition score, to allow comparison of the R^2.

Overall correlation	p-value	R^2
0.398	<0.001	0.159

Variable	Canonical loading
Set 1	
WMH	0.169
BG PVS dichotomised (UQ vs lower quartiles)	-0.014
CSO PVS dichotomised (UQ vs lower quartiles)	-0.042
PSMD	0.345
LVD	0.098
WB CMB present vs. absent	0.077
Lacune present vs. absent	0.125
Set 2	
Attention and processing speed	-0.874
Language	-0.521
Executive function	-0.771
Visuospatial function	-0.569
Memory	-0.761
Verbal memory	-0.682

Overall correlation	p-value	R^2
0.361	<0.001	0.130

Variable	Standardised regression coefficient
Set 1	
WMH	-0.437
BG PVS dichotomised (UQ vs lower quartiles)	0.048
CSO PVS dichotomised (UQ vs lower quartiles)	0.058

PSMD	-0.885
LVD	-0.242
WB CMB present vs. absent	-0.197
Lacune present vs. absent	-0.283
Set 2	
Global Cognition	1.00

Abbreviations: WMH – white matter hyperintensity; PV – periventricular; DW – deep white; WB – whole brain; CMB – cerebral microbleeds; PVS – perivascular space; BG – basal ganglia; CSO – centrum semiovale; UQ – upper quartile; LVD – large vessel disease; PSMD – peak skeletonised mean diffusivity.

Supplemental Table 5.5: Univariate associations of MRI markers with Global Cognition

Global Cognition	B	95.0% CI for B		SE B	R^2	Adj. R^2	Significance
		LL	UL				
WMH total volume	-0.01	-0.02	0.00	0.00		0.02	0.00
DW volume	-0.17	-0.03	-0.01	0.01		0.02	0.01
PV volume	-0.16	-0.03	0.00	0.01		0.02	0.01
Both DW and PV volumes together [a]					0.02	0.02	
PV Fazekas total	-0.14	-0.28	0.01	0.07		0.01	0.06
DW Fazekas total	-0.18	-0.32	-0.03	0.07		0.01	0.02
PV Fazekas – dichotomised ≥ 3	-0.29	-0.54	-0.04	0.13		0.01	0.02
DW Fazekas – dichotomised ≥ 3	-0.35	-0.65	-0.06	0.15		0.01	0.02
Fazekas total combined, as single variable (0-6)	-0.09	-0.17	-0.01	0.04		0.01	0.02
Both Faz PV and Faz DW scores together [a]					0.01	0.01	
CMB							
Continuous whole brain	-0.04	-0.09	0.01	0.03		0.00	0.13
Continuous deep	-0.19	-0.39	0.02	0.10		0.01	0.07
Continuous whole lobar	-0.05	-0.13	0.03	0.04		0.00	0.19
Dichotomised WB (1 vs 0)	-0.25	-0.55	0.05	0.15		0.01	0.10
Dichotomised deep (1 vs 0)	-0.54	-1.14	0.06	0.31		0.01	0.08
Dichotomised lobar (1 vs 0)	-0.18	-0.49	0.14	0.16		0.00	0.27

Trichotomised WB (2 vs 1 vs 0)	-0.16	-0.34	0.03	0.10	0.00	0.11
Multiple WB (≥2 vs 0/1)	-0.26	-0.66	0.14	0.20	0.00	0.21
Trichotomised deep (2 vs 1 vs 0)	-0.36	-0.74	0.02	0.19	0.01	0.06
Multiple deep (≥2 vs 0/1)	-0.72	-1.56	0.12	0.43	0.01	0.09
Trichotomised lobar (2 vs 1 vs 0)	-0.11	-0.31	0.09	0.10	0.00	0.29
Multiple lobar (≥2 vs 0/1)	-0.17	-0.61	0.28	0.23	0.00	0.46
PVS						
BG PVS continuous	0.00	-0.03	0.03	0.02	0.00	0.84
CSO PVS continuous	0.03	-0.01	0.06	0.02	0.00	0.16
BG PVS dichotomised (UQ vs lower quartiles)	-0.07	-0.35	0.21	0.14	0.00	0.64
CSO PVS dichotomised (UQ vs lower quartiles)	0.05	-0.22	0.32	0.14	0.00	0.74
PVS severe dichotomised in BG or CSO	0.04	-0.20	0.27	0.12	0.00	0.77
PVS severe dichotomised in both areas	-0.16	-0.60	0.28	0.22	0.00	0.47
Lacune						
Lacune present vs. absent	-0.38	-0.80	0.03	0.21	0.01	0.07
Large vessel disease						
LVD present vs. absent	-0.26	-0.75	0.23	0.25	0.00	0.30
DTI						
MD	-10269.49	-13723.84	-6815.14	1757.25	0.08	0.00
FA	11.14	5.96	16.31	2.63	0.04	0.00
PSMD	-4517.95	-5940.13	-3095.76	723.48	0.09	0.00
zPSMD	-0.35	-0.46	-0.24	0.06	0.09	0.00
lgPSMD	-4.68	-6.11	-3.25	0.73	0.09	0.00

Abbreviations: WMH – white matter hyperintensity; PV – periventricular; DW – deep white; WB – whole brain; CMB – cerebral microbleeds; PVS – perivascular space; BG – basal ganglia; CSO – centrum semiovale; UQ – upper quartile; LVD – large vessel disease; DTI – diffusion tensor imaging; MD – mean diffusivity; FA- fractional anisotropy; PSMD – peak skeletonised mean diffusivity.

B = unstandardized regression coefficient; CI = confidence internal; LL = lower limit; UL = upper limit; SE B = standard error of the coefficient; R2 = coefficient of determination; Adj. R^2 = adjusted R^2

[a] – Both variables entered into a multivariable equation.

Supplemental Table 5.6: Alternative CVD Index - multiple regression result for Global Cognition, excluding cerebral microbleeds (Sydney Memory and Ageing Study, imputed data N=421).

Global Cognition	B	95.0% CI for B LL	95.0% CI for B UL	SE B	β	R^2	Adj. R^2
Model						0.09	0.08
Constant	2.08	1.28	2.87	0.40			
Whole brain WMH volume (cm3)	0.01	-0.00	0.02	0.00	0.10		
Presence or absence of lacunes	-0.12	-0.53	0.28	0.21	-0.03		
PSMD	-0.54	-0.74	-0.34	0.10	-0.35***		

B = unstandardized regression coefficient; CI = confidence internal; LL = lower limit; UL = upper limit; SE B = standard error of the coefficient; β = standardised coefficient; R2 = coefficient of determination; Adj. R^2 = adjusted R^2

* p < 0.05; ** p < 0.01; ***p < 0.001

Supplemental Table 5.7: Summary table: MRI indices of CVD

Study lead author	Characteristics	MRI markers of CVD	Outcome measure(s)
Charidimou et al.[181]	105 ppts with CAA	7-point SVD ordinal scale (0-6) based on: Lobar CMB; cortical superficial siderosis; centrum semiovale dPVS; WMH.	Retrospective cross-sectional assoc. with CAA-associated vasculopathic changes and symptomatic intracerebral haemorrhage
Chuang et al.[217]	62 vascular cognitive impairment and dementia patients.	7-point SVD ordinal scale (0-6) based on lacune, microbleed, and WMH severity.	Cross sectional association with grey and white matter volumes, blood pressure and carotid flow velocity.
Dickie et al.[222]	288 ppts in 3 cohorts of mild stroke, SLE and healthy volunteers.	"Brain health index" -combined neurodegenerative and neurovascular pathology. Voxels divided into healthy or pathological.	Association with Staals SVD score and cognitive performance.
Gomez-Choco et al.[218]	4424 stroke patients	3-level scale based on severity of WMH, presence of lacune, presence of microbleed.	Function and neurological recovery after acute ischaemic stroke (on discharge)
Jokinen et al.[224]	560 older adults with mild to moderate WMH	A combined z-score based on volumetric measures of WMH, lacunes, enlarged perivascular spaces, chronic cortical infarcts, and global and regional brain atrophy.	Associations with longitudinal cognitive and functional outcomes.
Kandiah et al.[182]	209 ppts with mild stroke	15-point risk score based on: age, education, acute cortical infarcts, WMH, lacunes, global cortical atrophy, and large vessel stenosis	Post-stroke cognitive impairment at 6 month follow up
Koton et al.[219]	907 stroke-free ppts	3-level scale of 'microvascular brain disease', based on severity of WMH and presence of lacunes.	Progression of microvascular brain disease and association with stroke

Staals et al.[184]	678 ppts from healthy ageing cohort	5-point SVD ordinal scale (0-4) based on: lacune; extensive WMH; CMB[a]; dPVS in the basal ganglia	Cross-sectional assoc. with global cognition, processing speed and memory
Van Slotten et al.[225]	1,949 ppts without baseline dementia or depressive symptoms	Score based on 5 features: high WMH volume, brain parenchyma volume, subcortical infarcts, cerebral microbleeds, and large PVS	Association between score and incident depressive symptoms.
Verwer at al.[220]	90 memory clinic patients with MRI-defined vascular brain injury	4-point SVD ordinal scale (0-3) based on WMH severity and presence of lacunes and/or microbleeds.	Associations with "physical performance"; gait speed, balance, and chair stand performance
Wang et al.[221]	436 older ppts	4-point "microvascular lesion load" ordinal scale (0-3) based on WMH severity, presence of lacunes and PVS severity.	Associations with cognitive decline and incident dementia over 9 years.
Xu et al.[108]	305 cases and 94 controls from memory clinic	Weighted 4-category cerebrovascular disease burden score: None/very mild (0); Mild (1); Moderate (2) and Severe (3). Based on: cortical stroke; intracranial stenosis; multiple lacunes; multiple CMB; moderate to severe WMH.	Cross-sectional assoc. with global cognition and specific cognitive domains

[a] There have been modifications to this scale. In the original scale, a point was given for the presence of a deep microbleed. This was later changed to the presence of a bleed anywhere in the brain.

Abbreviations: WMH – white matter hyperintensity; PV – periventricular; DW – deep white; WB – whole brain; CMB – cerebral microbleeds; PVS – perivascular space; BG – basal ganglia; CSO – centrum semiovale; CAA – cerebral amyloid angiography; SLE -Systemic Lupus Erythematosus.

Supplemental Table 5.8: Additional multiple regression results

Supplemental Table 5.8a: CVD Index – multiple regression result for Global Cognition (Older Adults Twin Study, imputed data, N=413).

CVD Index	B	95.0% CI for B LL	95.0% CI for B UL	SE B	β	R^2	Adj. R^2
Model						0.09	0.08
Constant	1.16	0.41	1.91	0.38			
Whole brain WMH volume (cm3)	-0.01	-0.02	-0.00	0.01	-0.14*		
Presence or absence of cerebral microbleeds	0.00	-0.26	0.26	0.13	0.00		
Presence or absence of lacunes	-0.22	-0.71	0.28	0.25	-0.04		
PSMD $*10^{-4}$ mm^2/s	-2.36	-4.01	-0.70	0.85	-0.18**		

Supplemental Table 5.8b: CVD Index – multiple regression result for Global Cognition (Renji Cohort, imputed data, N=171).

CVD Index	B	95.0% CI for B LL	95.0% CI for B UL	SE B	β	R^2	Adj. R^2
Model						0.28	0.26
Constant	1.59	-0.70	3.86	1.16			
Whole brain WMH volume (cm3)	-0.01	-0.01	0.00	0.00	-0.16		
Presence or absence of cerebral microbleeds	0.15	-0.50	0.79	0.33	0.04		
Presence or absence of lacunes	-0.18	-1.29	0.93	0.57	-0.03		
PSMD $*10^{-4}$ mm^2/s	-6.32	-8.84	-3.79	1.29	-0.42***		

B = unstandardized regression coefficient; CI = confidence internal; LL = lower limit; UL = upper limit; SE B = standard error of the coefficient; β = standardised coefficient; R2 = coefficient of determination; Adj. R^2 = adjusted R^2

* p < 0.05; ** p < 0.01; ***p < 0.001

Chapter 6: Discussion.

6.1 Summary

This thesis set out to improve existing methods of CVD quantification. As described in the introduction, CVD is a common set of pathologies, especially in the elderly, and is responsible for a multitude of deleterious sequelae. In addition to strokes, cerebral haemorrhages and various neuropsychiatric symptoms, it is associated with vascular dementia, the second most common dementia subtype. There is also increasing recognition of the role CVD plays in worsening the risk and expression of AD. Given the lack of current disease modifying treatments for AD, modifying the vascular risk factors associated with CVD and AD is essential.

Accurate quantification of CVD burden is vital, in part because clinically, for an accurate diagnosis of vascular cognitive impairment or dementia to be made, a determination needs to be made as to whether a patient's cognitive deficits can be explained by the current CVD burden.

Current methods of neuroimaging CVD quantification are not optimal, however, in part due to the challenge of integrating multiple different lesion types, which can represent similar and overlapping aetiologies. In the introduction, existing methods of CVD quantification including multi-modal CVD indices were critiqued, and it was suggested that further research was needed to investigate the significance of individual MRI markers. While promising first steps have been made in the development of a CVD index, more refinement is needed, including consideration of non-structural imaging modalities.

Chapter 2 examined the association of CMB, an under-researched marker of previous micro-haemorrhages with cognitive impairment and incident dementia. I found a cross-sectional association with impaired executive function, which was not sustained longitudinally, whereas CMB were associated with a decline in visuospatial function. These associations were independent of the effect of other

neuroimaging measures. There were some regional differences when those with only a strictly lobar distribution of CMB were assessed.

After a reliable PVS rating scale was developed, as presented in Chapter 3, this was used to examine the associations of PVS with cognitive impairment and incident dementia in Chapter 4. I found that individuals with severe PVS pathology in the BG and the CSO regions, or in the CSO alone had greater decline in global cognition over four years and that for all PVS groups examined, there was an increased risk of incident dementia mid-study (i.e., years 4 to 6).

The CVD index was developed and validated against Global Cognition in Chapter 5. CMB and PVS added very little to the proportion of variance explained. PVS were not included in the final model and the presence or absence of CMB at baseline added little in the presence of WMH volume and PSMD. The index performed well in the development cohort, explaining 9% of the proportion of variance, but performance was variable when validated in separate cohorts, with R^2 ranging from 5 to 13%, likely due to the differing characteristics of the cohorts.

Despite this, it performed better than the most widely used CVD index, developed by Staals et al[107] and compared favourably with WMH volume, the most established SVD measure. PSMD was the strongest individual predictor of Global Cognition in all three cohorts and had a greater R^2 than the index in the validation cohorts, i.e., 0.25 in Renji. My results suggest that the premise that a multi-modal index is more informative than a single measure (the primary hypothesis of the thesis), is valid and the inclusion of non-structural DTI measures is valuable, but the challenge is developing an index that performs equally well in diverse cohorts. Specifically, the weightings derived from the MAS cohort did not apply equally to heterogenous validation cohorts with differing characteristics.

My results support secondary hypothesis 1; that the Index shows a stronger association with Global cognition than the Staals et al[107] index as well as hypothesis 2: that the performance of the Index improved with the inclusion of the DTI-measure PSMD.

6.1 Overarching themes

Several themes emerged when exploring the quantification of CVD.

The heterogeneity of rating techniques and scales for SVD markers:

There are several rating scales used to visually rate CMB[68, 308, 309] and the appearance of CMB also depends of MRI field strength and imaging parameters[383]. In relation to PVS, there are a greater number of rating methods examining this feature, with 12 described in Table 3.1 in Chapter 3. These cover diverse methods examining global PVS, PVS in representative slices, or in up to 4 subregions where they commonly occur. This heterogeneity of scales has led to very different prevalence rates and associations with cognition. The STRIVE[38] criteria, published in 2013 were an important attempt to standardise the delineation of SVD, but this remains a major challenge for the field. It is hoped that automated rating of some of these lesions may improve this problem, but automation may add another source of heterogeneity. For examples there are numerous supervised, unsupervised and semi-supervised segmentation methods for WMH[384], which can produce variable results[385].

Benefits of regional measures:

I found that for CMB and PVS sub-regions, there were differential associations with impairments in specific cognitive domains, global cognition and incident dementia. Lobar CMB and CSO PVS are thought to represent CAA or mixed CAA/hypertensive pathology, whereas BG PVS and deep CMB, are more likely to be related to hypertension[329, 386]. Further, associations with cognition may depend on the WM tract affected by the lesion, with a recent review finding certain strategic white matter tracts such as the forceps minor or anterior thalamic radiation (ATR) being important[149]. A recent paper by our group mapped white matter lesions onto specific tracts and showed that regional WMH lesions were associated with impairments in cognition, independent of the total white matter lesion load[47]. The mechanisms leading to cognitive impairment are complex however and local disruption may lead to less efficient structural and global networks. These network

perturbations have been shown to be a mediator for the effect of SVD on cognition[91, 92], and is a potentially productive field for future research.

I did not include any regional markers in the final index however and this may be a reflection on limited sample size and power when examining sub-regions. Further exploration of region-based approaches[149] is needed, with large samples to investigate lesions in multiple regions.

Differences between cross-sectional and longitudinal results:

For the individual MRI lesions examined, I found differences between cross-sectional and longitudinal results. With CMB, there was a cross-sectional association with poor executive function but longitudinal cognitive decline showed an association only with visuospatial ability. For individuals with severe CSO PVS pathology, there was a cross-sectional association with impaired memory, but not with global cognition. Longitudinally, however, there was a decline in global cognition, but not with any specific cognitive domain, including memory. This raises two issues. First, that neuropsychological tests are multifactorial in structure and even though a test may be designed to focus on one aspect of cognition, test performance involves multiple cognitive processes [319]. We cannot always delineate between cognitive domains cleanly and this lack of clear delineation between different cognitive domains is one of the reasons for the complex and often contradictory results in the published literature. Visuospatial ability for example, was assessed through Block Design, but this test incorporates a range of other cognitive abilities, including aspects of executive function and processing speed which are a particularly important contributor to performance above age 75 [317, 318]. As discussed in Chapter 2, when a principal component analysis was previously performed on the MAS data, the first principal component comprised attention/processing speed, executive function and visuospatial factors, suggesting significant correlations between the measures used.

Second, that cross-sectional and longitudinal data may represent separate but overlapping processes. In the cohort studies used in thesis, there may be less variability in cross-sectional cognition, as participants at baseline will have been

screened to exclude those with symptomatic pathology and they are younger, with less prevalence of cognitive impairment. In contrast, as participants age and have greater risk for cognitive decline, this generally leads to greater variability. Longitudinal change in cognition arguably demonstrates stronger associations with neuroimaging measures. Cross-sectional measures do not account for baseline characteristics of the measures including previous disease, even when age, education, medical history and other variables are taken into consideration. Longitudinal change is intra-individual and therefore better able to account for individual variation. Further, at baseline, lesions may not be symptomatic but this may change over time, as the mediating effect of cognitive reserve[387] changes.

Variable relationship with time:

One unexpected result from the longitudinal PVS study was the interaction of time with the impact of PVS on cognition. The strongest associations were mid-study, at years 4-6, but this effect was not sustained at year 8. I speculate this to be due to the increased number of incident dementia cases around year 8, overwhelming the signal from PVS. The interaction with time is not commonly discussed in the CVD literature but can explain some of the divergent results seen with longitudinal cognitive changes and incident dementia. It needs to be considered in future studies.

6.2 Limitations and challenges

The challenges of assessing CVD via neuroimaging have been discussed throughout the thesis extensively. To reiterate briefly, there are multiple cerebrovascular lesion types that may represent overlapping pathologies and occur at different time points on a continuum. These lesions are often common, particularly in the elderly and defining a threshold to label them as pathological is not always clear-cut, especially as their quantification is technology dependent, and may vary by MRI field strength and imaging parameters. Once visualised, their quantification is generally not standardised and will differ according to rating scale, or automated method.

In ageing studies, attrition is common, producing significant missing data. Traditional analytic studies often assume data are missing at random, but in the study cohorts, I unsurprisingly found non-completers are more likely to be unwell or cognitively impaired [386]. This is consistent with other ageing studies [368, 369]. I used a variety of analytic strategies to account for this, including sensitivity analyses, but it is an ongoing challenge which may bias results, including producing type-II errors.

Finally, when considering the overall burden from CVD, choice of the validation measure needs careful consideration. I chose Global Cognition rather than specific cognitive domains for the index, a decision that was supported by the canonical correlation analysis. Although executive function and attention and processing speed are domains commonly affected with VCD, any domain can be impacted and there is no pathognomonic pattern of cognitive impairment[1]. It is also difficult to disaggregate the contribution of different pathologies, which may include neurodegenerative pathology. An alternative of validation could be incident vascular dementia diagnosis, but this requires longer study durations and is more susceptible to attrition. When validated against neuropathology, the clinical diagnosis of possible VaD is relatively inaccurate, with an average sensitivity of 0.49 (range 0.20–0.89) and an average specificity of 0.88 (range 0.64–0.98)[388]. This is further complicate by the fact that, unlike AD or Lewy body disease, there are no widely accepted criteria for neuropathological diagnosis of VaD or VCI[119] , as discussed in the systematic review above[2]. These uncertainties bedevil the field currently and their resolution needs to await developments in neuropathology, neuroimaging and non-imaging markers of VaD.

6.3 Future directions

There is significant scope to further develop the novel CVD index. Given the difficulty of contextualizing an individual score, I plan to automate the index as an online calculator. Index scores can then be compared to age- and gender- matched participants in the development cohort. This would also save the clinician or researcher time. The automation will facilitate its use by other research groups and clinicians and cumulative data can be used to refine the index continuously.

The rating of certain SVD measures, particularly CMB and PVS, is time-consuming, requires training and experience and is laborious. There have been multiple attempts to automate the quantification of CMB[389, 390] and PVS[335, 336], but no method is ready for widespread clinical used. When these methods mature, they can improve intra-rater reliability and will speed up the quantification of CVD and associated indices.

The index should be validated with other phenotypes of interest. Predicting longitudinal cognitive change and incident dementia are clinically vital outcomes. Expanding the range of included cohorts in the index would be beneficial, so I can both refine the model and validate it across a range of populations. A larger sample should also provide enough power to further investigate region-based approaches.

Finally, other MRI modalities should be considered for inclusion in any future index, particularly non-structural methods, such as cerebral reactivity approaches[391], network analysis[90, 91] and perfusion imaging. Arterial spin labeling[381] provides perfusion information on a global or localized scale and changes in regional brain function may show greater sensitivity to early disease, disease progression, or responses to therapy than changes in traditional structural MRI volumetrics [381, 382].

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