Blood-Brain Barrier Imaging in Cerebral Small Vessel Disease

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A thesis submitted for the degree of
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Cerebral small vessel disease (cSVD) refers to a group of conditions associated with impaired functioning of the smallest blood vessels supplying the brain. Whilst cSVD causes around a quarter of all strokes and contributes to approximately half of all cases of dementia – as well as disturbances in gait, mood and cognition - the condition is often overlooked and treatment options are limited. We found evidence of white matter hyperintensities (WMHs), often indicative of cSVD, on over 40% of routinely performed brain scans locally. Recent evidence suggests that changes in the highly specialised blood-brain barrier (BBB) underlie the early pathophysiology of cSVD. Measuring water exchange across the BBB using magnetic resonance imaging may be a sensitive marker for BBB dysfunction. Our systematic scoping review identified 38 clinical BBB water exchange studies in a number of disorders, with seven focussed on cSVD. Studies were heterogeneous, as expected with exploratory research, but with encouraging trends across several pathologies including measures of repeatability. We performed an exploratory study of filter-exchange imaging (FEXI) in patients with cSVD, compared with healthy volunteers. Whilst the imaging protocol was well tolerated, there was no significant difference between BBB water exchange between groups nor correlation with WMH volume. However, we demonstrate important associations between WMH volume, cognition and QRISK3 as well as an interesting difference in extra-vascular diffusivity (thought to reflect altered microstructural integrity) between groups. Larger, longitudinal studies are required to determine the sensitivity of these novel measurements as markers of cSVD and to assess their prognostic value for clinical parameters such as cognitive decline.

Overview of Chapters:

Chapter 1 of this thesis will outline the prevalence, pathophysiology, clinical manifestations and management of cSVD before focussing on the role of the blood-brain barrier (BBB) and how to measure BBB function (*or dysfunction*).

Chapter 2 will utilise routinely collected data to understand our local cSVD population and examine for trends in age, performance, levels of deprivation, vascular risk factors, co-morbidity and radiological findings.

Chapter 3 will look ahead at the emerging field of Magnetic Resonance Imaging (MRI) based BBB water exchange imaging, with a systematic scoping review of the currently published clinical studies.

Chapter 4 will define the protocol I have written for a for an exploratory clinical study utilising Filter-EXchange Imaging (FEXI) as a measure of BBB water exchange in a small cohort of patients with cSVD compared to healthy controls. **Chapter 5** will report the results of this NIHR portfolio adopted study.

Chapter 6 will discuss the findings and impact of this PhD thesis in the context of the current literature and make recommendations for future study.

Acknowledgements:

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I am sincerely grateful to my parents for their unconditional love and support. Thank you to my neurology registrar colleagues, in particular to Dr Jasmine Wall for her expertise in academic and statistical methodology, my friend Dr Edward Bezant for his own personal brand of encouragement (and for booking the squash court every week) and to the wonderful minds I have had the pleasure of meeting within the D33/CHICAS team.

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This thesis is dedicated to my wife, *Megan* and our two beautiful children

Sienna and *George*.

Follow your dreams.

Declaration:

I declare that the work presented in this thesis is my own work and has not been submitted, either in whole or in part, for a degree at this, or any other university.

Sections of this thesis which have been published elsewhere in peer-reviewed journals or presented at conferences have been identified clearly and may be formatted according to the journal requirements.

I have referenced the contribution of others, where relevant.

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Manuscripts:

- Jackson M, Szczepaniak M, Wall J, **Maskery M**, Mummery C, Morrish P, Williams A, Knight J, Emsley HCA. Numbers and types of neurological emergencies in England and the influence of socioeconomic deprivation: a retrospective analysis of hospital episode statistics data. BMJ Open. 2022.
- Powell E, Dickie BR, Ohene Y, **Maskery M**, Parker G, Parkes LM. Blood-brain barrier water exchanger measurements using contrast-enhanced ASL. NMR in Biomedicine. 2023.
- **Maskery MP**, Whittam D, Nawaraj S, Chakraborti S, Arunachalam C, Munavvar M, Shaik S. Myeloperoxidase-positive ANCA-associated vasculitis presenting as myalgia, proximal weakness and a normal CK. Practical Neurology. 2023.
- *[In peer review, pre-print online]* **Maskery MP**, Rennie N, Mathur S, Knight J, Emsley HCA. Prevalence of radiological cerebral small vessel disease: an insight from routinely collected data. BMC Neurology. 2024.

Poster presentations:

- **Maskery MP**, Mathur S, Knight J, Emsley HCA. Prevalence of radiological cerebral small vessel disease in the regional 2-week wait direct to scan MDT. Lancaster University Postgraduate Symposium, Lancaster. 2023.
- **Maskery MP**, Mathur S, Knight J, Emsley HCA. Prevalence of radiological cerebral small vessel disease in the regional 2-week wait direct to scan MDT. Association of British Neurologists Annual Meeting, Belfast. 2023 *[included in poster tour]*.

Invited presentations:

- How can vasculitis affect me? Vasculitis Patient Awareness Evening. Health Innovation Campus, Lancaster University. March 2024.
- Blood-brain barrier imaging in cerebral small vessel disease. Red Rose Research (3Rs) Medical Meeting. Preston North End (Deepdale) Stadium. June 2024.

Abbreviations:

2WW	2 Week Wait
AD	Alzheimer's Disease
ASL	Arterial Spin Labelling
ATT	Arterial Transit Time
AVM	Arteriovenous Malformation
BBB	Blood-Brain Barrier
BECs	Brain Endothelial Cells
BMET	Brief Memory and Executive Test
CAA	Cerebral Amyloid Angiopathy
CADASIL	Cerebral Autosomal Dominant Arteriopathy Subcortical Infarcts and Leukoencephalopathy
CARASIL	Cerebral Autosomal Recessive Arteriopathy Subcortical Infarcts and Leukoencephalopathy
CBF	Cerebral Blood Flow
CI	Confidence Interval
CNS	Central Nervous System
COVID-19	Coronavirus Disease 2019 (SARS-CoV-2)
CRF	Clinical Research Facility
CSF	Cerebrospinal Fluid
cSVD	Cerebral Small Vessel Disease
CT	Computed Tomography
DCE-MRI	Dynamic Contrast Enhanced Magnetic Resonance Imaging
eGFR	Estimated Glomerular Filtration Rate
ENT	Ears, Nose and Throat (Otolaryngology)
EPSRC	Engineering and Physical Research Council
ESO	European Stroke Organisation
EST	Education, Skills and Training Deprivation
FA	Fractional Anisotropy
FEXI	Filter Exchange Imaging
GBCAs	Gadolinium Based Contrast Agents
GDPR	General Data Protection Regulation

GM	Grey Matter
GP	General Practitioner
HDD	Health Deprivation and Disability
HRA	Health Research Authority
ICC	Intraclass Correlation Coefficient
IDEALS	Intrinsic Diffusivity Encoding of Arterial Labelled Spins
IgG	Immunoglobulin G
IIH	Idiopathic Intracranial Hypertension
IMD	Index of Multiple Deprivation
LACI-2	The Lacunar Intervention Trail-2
LP	Lumbar Puncture
LSOA	Lower Layer Super Output Areas
LTH	Lancashire Teaching Hospitals
MarkVCID	Biomarkers for Vascular Contributions to Cognitive Impairment and Dementia Consortium (study)
MCI	Mild Cognitive Impairment
MD	Mean Diffusivity (measured in Diffusion Tensor Imaging)
MDT	Multi-disciplinary Team
MELAS	Mitochondrial Encephalopathy Lactic Acidosis and Stroke-like episodes
MMPs	Matrix Metalloproteinases
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging *T1w: T1-weighted:* *T2w: T2-weighted:* *FLAIR: Fluid Attenuated Inversion Recovery* *SWI: Susceptibility Weighted Imaging* *ADC: Apparent Diffusion Coefficient* *DWI: Diffusion Weighted Imaging*
MS	Multiple Sclerosis: *RRMS: Relapsing Remitting MS* *PPMS: Primary Progressive MS* *SPMS: Secondary Progressive MS* *PMS: Progressive MS*

NAGM	Normal Appearing Grey Matter
NAWM	Normal Appearing White Matter
NHS	National Health Service
NIHR	National Institute for Health and Care Research
NMO	Neuromyelitis Optica
NVU	Neurovascular Unit
PET	Positron Emission Tomography
PHQ-2/-9	Patient Health Questionnaire
PI	Principle Investigator
PIS	Participant Information Sheet
PLD	Post Labelling Delay
PRISMA-ScR	Preferred Reporting Items for Systematic Reviews and Meta-Analysis – Scoping Review
R4VaD	Rates, Risks and Routes to Reduce Vascular Dementia (study)
REC	Research Ethics Committee
RF	Radiofrequency
ROI	Region of Interest
ROS	Reactive Oxygen Species
SNR	Signal to Noise Ratio
SPECT	Single Positron Emission Computed Tomography
STRIVE	Standards for Reporting Vascular Changes on Neuroimaging
T	Tesla (field strength in magnetic resonance imaging)
TIA	Transient Ischaemic Attack
U&E	Urea & Electrolytes
UBO	'Unidentified Bright Object' *(no longer favoured terminology)*
UoM	University of Manchester
WEPCAST	Water Extraction with Phase Contrast Spin Tagging
WEX-BRAIN	Water Exchange in the Vasculature of the Brain
WHO	World Health Organisation
WM	White Matter
WMH	White Matter Hyperintensity

Prologue:

Doctor: ‘Your brain scan is normal’

Patient: ‘Thank goodness! Completely normal?’

Doctor: *[shrugs]* ‘Well, it’s perfectly normal for a gentleman *of your age.’*

Here began my fascination with what I have affectionately, though rather non-mellifluously, termed the ‘non-specific white matter hyperintensity shrug’. Thankfully, this gentleman’s magnetic resonance imaging (MRI) scan had indeed excluded evidence of brain malignancy, large artery infarct and intracranial haemorrhage and the report had been concluded with the welcome phrase ‘no acute intracranial abnormalities detected’. Cue a sigh of relief, a discharge summary and a briefly empty hospital bed.

However, reference had also been made within the scan report to ‘age commensurate T2-weighted white matter hyperintensities (WMHs) likely in keeping with chronic ischaemic change’. Notice that mention of these had been omitted from the dialogue above. I have encountered similar – but certainly not consistent - wording all too often. Whether these WMHs were described as ‘cerebral small vessel disease’ (cSVD), ‘microvascular change’ or in a report by a more experienced colleague to simply say the words ‘UBOs [Read: ‘Unidentified Bright Objects’] – Binswanger’s Disease’, they were all probably referring to the same spectrum of radiological abnormalities.

We know that the majority of these WMHs can be attributed to changes in the smallest blood vessels within the brain, namely cSVD, but they can also be a sign of central nervous system demyelination – for example, multiple sclerosis – as well as being linked with migraine, epilepsy, head injury and somewhat nihilistically with the torment of *normal* ageing. Changes

in the specialised structure known as the blood-brain barrier (BBB) are thought to be central to the underlying disease process and imaging findings.

Differentiating between these potential underlying aetiologies based upon subjective analysis of WMH morphology and topographical distribution is challenging, particularly when the radiological burden is low or the clinical picture is mixed. Furthermore, in practice scans are interpreted by a radiologist in the context of a clinical vignette (of varying quality), which may consciously or unconsciously introduce bias. It is therefore not uncommon to receive a report of 'non-specific' or, much maligned, 'equivocal' WMH changes which can lead to uncertainty, unnecessary investigation and delayed diagnosis.

There is said to be little clinico-radiological correlation between symptom severity and the conventional imaging burden of WMHs. Seemingly, patients may be entirely asymptomatic. However, we know already that WMHs in the context of cSVD can present with an often-overlooked clinical syndrome including cognitive dysfunction, reduced mobility, incontinence, depression and loss of independence. Indeed, cSVD is responsible for almost half of dementias and a quarter of all strokes.

With these currently unsolved challenges, it is often difficult to communicate the presence of WMHs effectively to patients – particularly when the changes are present *incidentally* (or 'covertly') upon imaging performed for other reasons. There is also no specific disease modifying therapy, with consensus-based management guidelines limited to measures recommended for general cardiovascular health. Future interventional studies will rely upon sensitive, reproducible, cost-effective surrogate markers of cSVD associated with clinically meaningful end points, for which there is increasing focus on measuring BBB dysfunction.

Chapter 1: Cerebral small vessel disease and the blood-brain barrier.

Contribution statement:
I am responsible for the design, drafting and amendments to this literature review.

Distribution:
Not applicable.

Introduction:

Cerebral small vessel disease (cSVD) refers to a heterogeneous spectrum of pathological, clinical and radiological presentations relating to the structure and function of the smallest blood vessels within the brain.[1] It is one of the most prevalent conditions encountered by clinicians.[2] Whilst commonly considered a disease of older patients and related to vascular risk factors, there are several independent early-life predictors of cSVD severity including birth weight and education level.[3-6]

Clinically overt cSVD accounts for 25% of all strokes, in the form of lacunar infarcts or intracerebral haemorrhage, and approximately 50% of all dementia diagnoses.[7] However, the majority of cSVD manifests radiologically in potentially asymptomatic patients. When covert cSVD is present in the context of acute stroke or Alzheimer's disease, the unfavourable prognosis is compounded.[8-10] Often overlooked, cSVD can manifest with insidious cognitive impairment, gait deterioration or cerebrovascular parkinsonism leading to falls, urinary incontinence and a range of neuropsychiatric presentations from apathy to depression.[11,12] Late onset epilepsy has also been associated with cSVD.[13] The personal and wider economic burden of cSVD, in the form of loss of independence, institutionalisation and healthcare requirements cannot be overstated.[14]

Aetiopathogenic classification of cSVD:

A simplified aetiopathogenic classification system has been proposed for the diverse spectrum of cSVD, divided into 6 phenotypes (Table 1-1).[15]

Type 1 cSVD is due to arteriolosclerosis and probably represents the majority of cases, often referred to as 'sporadic' and is related to vascular risk factors such as hypertension.[16,17] The striking loss of normal blood vessel architecture in this form of cSVD was described in Fisher's seminal post mortem work in 1965, due to the process of lipohyalinosis in brains from patients with severe hypertension and lacunar infarcts.[18] Atherosclerotic plaques, loss of smooth muscle cells in the tunica media and microaneurysm formation causing thickening of the vessel wall and narrowing of the lumen have been described subsequently.[19] The resultant increase in media-to-lumen ratio (see Figure 1-1) results in chronic hypoperfusion and ischaemia.

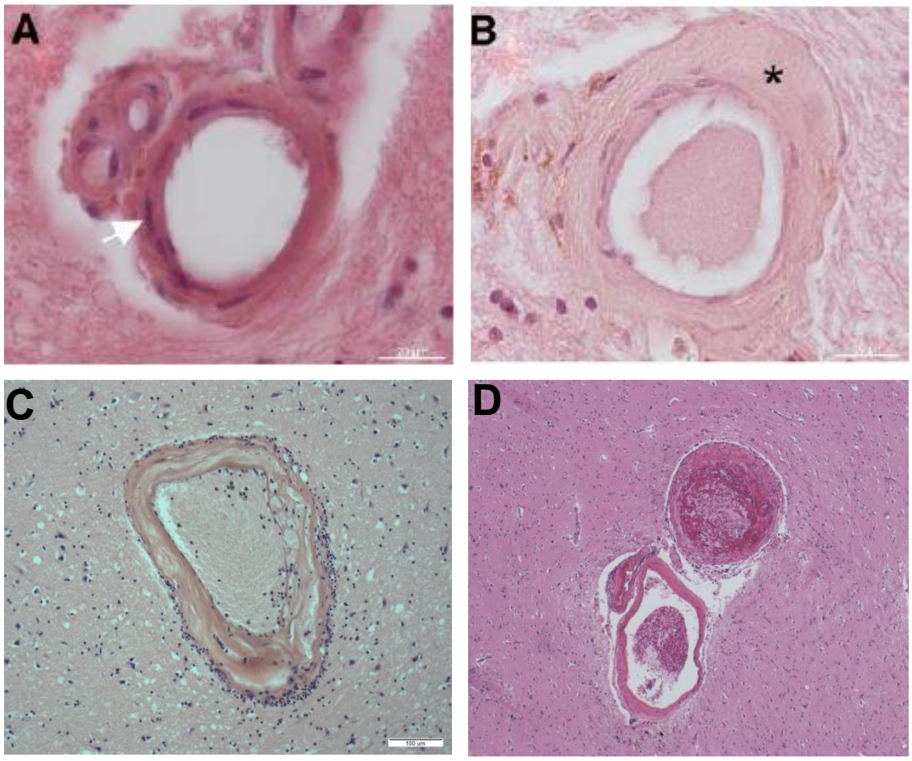


Figure 1-1: Small arterial blood vessels in human brain. (A) Normal, healthy penetrating artery with thin wall. (B) Arteriolosclerosis with approximately concentric wall thickening and *acellular hyaline material. (C) Lipohyalinosis with asymmetrical wall thickening around the lumen. (D) Fibrinoid necrosis. Haematoxylin and eosin (H&E) staining. Images (A) and (B) adapted from Hainsworth *et al.*[20] and (C) and (D) kindly supplied by Professor C Smith, University of Edinburgh.

Type 2 cSVD is increasingly recognised in the elderly population and refers to sporadic or hereditary cerebral amyloid angiopathy (CAA). CAA is characterised by deposition of amyloid-β peptide within the small cortical and leptomeningeal arteries and represents the most common cause of cerebral microbleeds and lobar intracerebral haemorrhage in the elderly.[21] Whilst the pathophysiology is not fully understood, there is considerable overlap with other amyloid pathologies such as Alzheimer's disease.[22]

Types 3-6 are significantly less prevalent. Despite an increasing number of single-gene or mitochondrial disorders associated with hereditary or genetic forms of cSVD (type 3), even collectively these are comparatively rare. For example, cerebral autosomal dominant

arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is due to a mutation of the NOTCH3 gene on chromosome 19q12 and represents the most common monogenic form of cSVD with a prevalence of only approximately 5 per 100,000.[23] The consensus recommendations for the management of monogenic cSVD have recently been published.[24]

Type 4 cSVD refers to an autoimmune or infection mediated process (for which the mainstay of initial management would be treatment of the underlying cause), type 5 due to venous collagenosis and type 6 due to microvessel degeneration in the context of factors such as radiotherapy. This review will focus on the most prevalent forms of cSVD, namely types 1 and 2.

Aetiopathogenic classification of cSVD
Type 1: Arteriolosclerosis Fibrinoid necrosis, lipohyalinosis, microatheroma, microaneurysms and segmental arterial disorganisation
Type 2: Sporadic and hereditary cerebral amyloid angiopathy Predominantly amyloid-β deposition, with smaller numbers related to vascular amyloid deposits of proteins such as amyloid-British protein (ABri), amyloid-Danish protein (ADan), gelsolin, prion protein (PrP), transthyretin (TTR) and cystatin C.
Type 3: Inherited or genetic small vessel diseases distinct from cerebral amyloid angiopathy CADASIL (NOTCH3) CARASIL (HTRA1) MELAS Fabry's disease Type IV collagen mutation related (COL4A1/COL4A2) Retinal vasculopathy with cerebral leukoencephalopathy (TREX1)
Type 4: Inflammatory and immunologically mediated small vessel diseases Immune mediated: Primary angiitis of the CNS Secondary CNS vasculitis Granulomatosis with polyangiitis Eosinophilic granulomatosis with polyangiitis Microscopic polyangiitis Hypersensitivity vasculitis Immunoglobulin A vasculitis Cryoglobulinaemic vasculitis Systemic lupus erythematosus CNS vasculitis Sjögren syndrome associated vasculitis Rheumatoid vasculitis Mixed connective tissue disease-associated vasculitis Behcet's vasculitis Infection medicated: e.g., menigovascular neurosyphilis, varicella zoster virus, cytomegalovirus, human immunodeficiency virus, fungus.
Type 5: Venous collagenosis
Type 6: Other small vessel diseases For example, post-radiation angiopathy and non-amyloid microvessel degeneration in Alzheimer's disease.

Table 1-1: Aetiopathogenic classification of cSVD, adapted from Pantoni *et al.* and Cannistraro *et al.*[3,15,25]

The specific underlying pathology of cSVD is heterogeneous and entangled with co-morbidity, environmental exposure and the normal ageing process.[1] Complicating matters further, it is difficult to visualise the small vessels *in vivo,* interpretation of animal models is limited and post mortem studies likely reflect an end-stage of the disease process.[26,27] However, a common theme of endothelial injury, impaired neurovascular coupling and chronic ischaemia leading to extravasation of blood products, inflammation, axonal damage and apoptosis, oligodendrocyte dysfunction and gliosis has emerged.[28,29] Increasingly, there is focus on the role of the blood-brain barrier (BBB) in the development and progression of a range of common neurological disorders including in the very early stages of cSVD pathogenesis.[30-34]

Diagnosis of cSVD:

Covert cSVD is often detected radiologically upon magnetic resonance imaging (MRI) performed during routine clinical practice.[35] Conventional surrogate markers of cSVD include white matter hyperintensities of presumed vascular origin (WMHs), perivascular spaces, cerebral microbleeds and lacunes (see Figure 1-2).[36] In 2013, Standards for Reporting Vascular Changes on Neuroimaging 1 (STRIVE-1) aimed to unify the definitions of radiological cSVD manifestations and encourage a consistent lexicon for routine reporting.[37] These recommendations were updated in 2023 with the publication of STRIVE-2 (see Table 1-2 for glossary of cSVD radiological findings).[38]

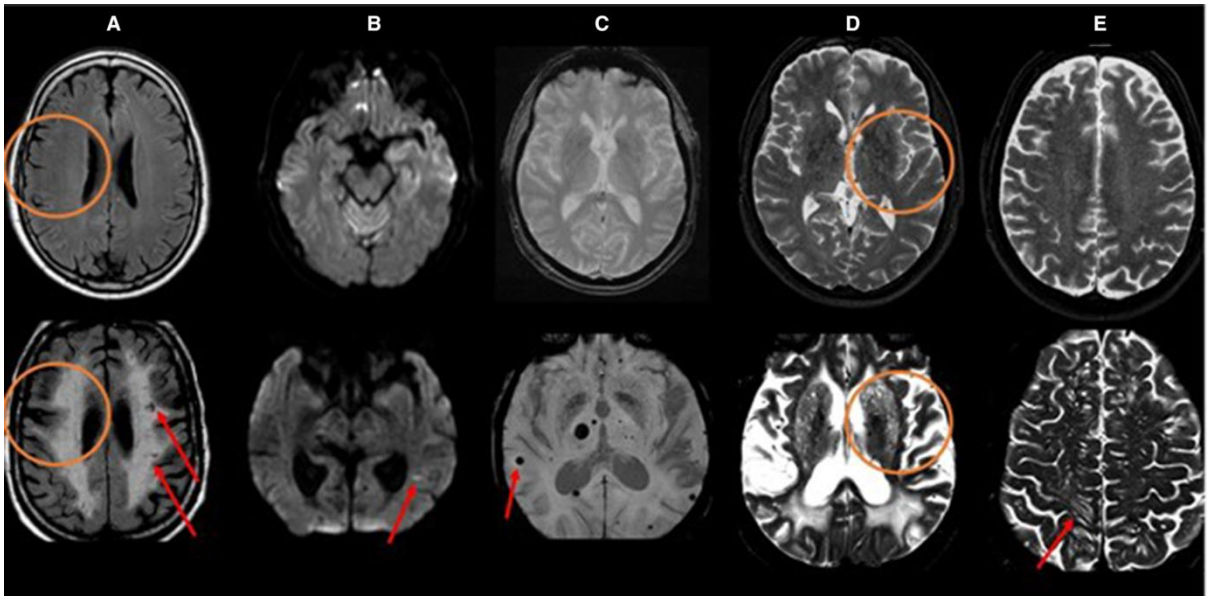
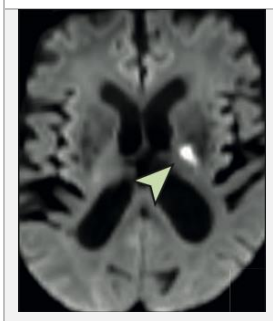
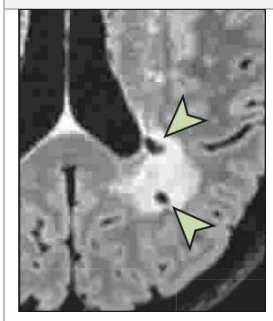
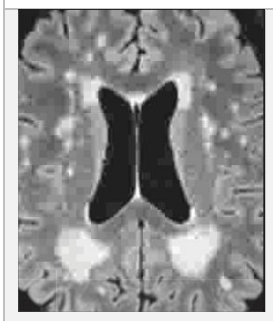
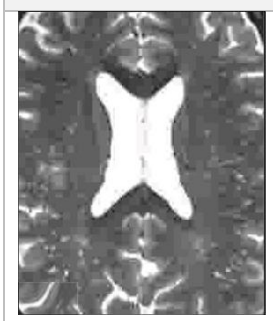
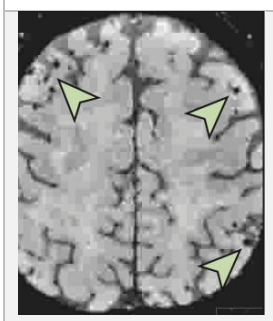


Figure 1-2: Cerebral small vessel disease markers on brain MRI. Top row – normal scan for comparison, bottom row – severely abnormal scan. A – FLAIR sequence showing severe WMH, with covert brain infarcts denoted by the arrows. B – DWI sequence showing a punctate infarct (arrow). C – T2* GRE (top) and SWI (bottom) showing multiple CMBs (arrow points to a single example). D – T2 sequence showing high burden on perivascular spaces in the basal ganglia region. E – T2 sequence showing high burden of perivascular spaces in the centrum semiovale region. Compare the circled area in the normal scan (top) and the severely abnormal scan (bottom). Taken from Hannawi *et al* (Creative Commons Attribution – NonCommercial-NoDerivs License).[39]

Glossary of proposed terms and definitions for neuroimaging features of cerebral small vessel disease:	
	**Recent small subcortical infarct** Neuroimaging evidence of a recent infarction in the territory of one perforating arteriole, with imaging features and clinical symptoms consistent with a recent lesion.
	**Lacune (of presumed vascular origin)** Round or ovoid, subcortical, fluid filed (similar signal to CSF) cavity up to 15mm in diameter that is likely to be end tissue damage from a recent small subcortical infarct, small subcortical haemorrhage, incidental diffusion-weighted-imaging-positive lesion, or end-stage cavitation in a white matter hyperintensity.
	**White matter hyperintensity (of presumed vascular origin)** Signal abnormality of variable size in the white matter that is hyperintense on T2-weighted images, such as fluid-attenuated inversion recovery (FLAIR), without cavitation (signal different from CSF). Lesions in the subcortical grey matter or brainstem are not included into this category unless explicitly stated - where deep grey matter and brainstem hyperintensities are included as well, the collective name should be subcortical hyperintensities.
	**Perivascular space** Fluid-filled space, which follows the typical course of a vessel penetrating the brain through grey or white matter; has signal intensity similar to CSF on all sequences; has a round, ovoid or linear shape (depending on the slice direction) with a diameter commonly not exceeding 2mm when imaged perpendicular to the course of the vessel.
	**Cerebral microbleed** Small (usually 2-5mm or sometimes 10mm in size) areas of signal void with associated blooming artefact on T2* or other MRI sequences sensitive to susceptibility effects.

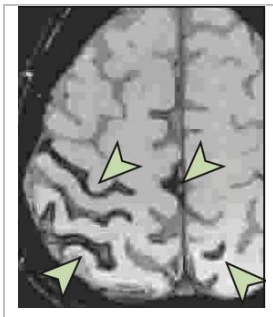
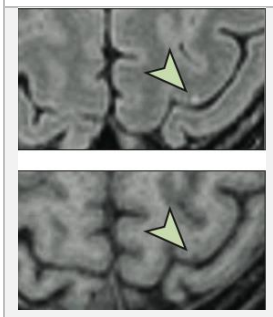
	**Cortical superficial siderosis** Thin areas of hypointensity on T2*, or other MRI sequences sensitive to susceptibility effects, in or overlying the superficial cortex, which can be confined to one gyrus or adjacent sulci, or occasionally more widespread affecting several brain regions.
	**Cortical cerebral microinfarct** Small lesions appearing hypointense on T1-weighted, hyperintense on T2-weighted of FLAIR, and isointense on T2*-weighted MRI, operationally defined to be strictly cortical and with an upper size limit of 4mm.
	Brain atrophy Brain volume loss, not related to a specific microscopic focal injury such as trauma or infarction; thus, the focal injury is not including in this measure unless explicitly stated.

Table 1-2: Conventional radiological markers of cSVD, adapted from the recent STRIVE-2 guide (with permission from Professor J Wardlaw, University of Edinburgh).[38]

Population studies have shown WMHs emerge even before middle age and increase in prevalence with age to be present upon >90% of scans performed in patients over 90 years old.[40] WMHs have been shown to be associated with vascular risk factors and to be a marker of BBB dysfunction.[41-43] Whilst histological correlation is limited, they appear to represent areas of impaired white matter structural integrity with neuronal loss and gliosis.[44,45] Contrary to previous opinion, WMHs have recently been shown to be dynamic; able to both progress and regress upon longitudinal imaging.[44]

Grading scales have been proposed to describe the burden of WMHs, such as the widely adopted Fazekas scale - based upon WMH location (periventricular vs deep WMHs) and their level of confluence (see Table 1-3).[46]

Periventricular white matter hyperintensities
0 – absence
1 – ‘caps’ or pencil-thin lining
2 – smooth ‘halo’
3 – irregular periventricular white matter hyperintensities extending into the deep white matter
Deep white matter hyperintensities
0 – absence
1 – punctate foci
2 – beginning confluence of foci
3 – large confluent areas

Table 1-3: The Fazekas scale.[46]

However, WMHs are not specific for cSVD and are associated with various alternative pathologies such as multiple sclerosis, traumatic brain injury, epilepsy, migraine and they have been shown to increase with age.[47-50] The morphological appearance and topographical distribution of WMHs can be used to help distinguish their underlying aetiology, but they remain an imprecise measure of cSVD.[51] A total cSVD score has been more recently proposed, considering the presence of lacunes, microbleeds and perivascular spaces as additional radiological markers (see Table 1-4),[36] with a higher score shown to be associated with lower general cognitive ability in a population of healthy older adults.[52]

MRI feature	Definition	Score
Lacunes	≥ 1 lacune	1 point
Microbleeds	≥ 1 microbleed	1 point
Perivascular spaces	Moderate to severe perivascular spaces in basal ganglia	1 point
White matter hyperintensities (WMH)	Periventricular WMH Fazekas 3 and / or deep WMH Fazekas 2-3	1 point

Table 1-4: Total cSVD score[36]

Management of cSVD:

In cSVD, management can either be directed towards clinically overt manifestations such as lacunar stroke/vascular dementia, clinical symptoms or to covert radiological disease. For CAA, the management is largely aimed at mitigating the risk of future intracerebral haemorrhage.[53] Specialist cSVD outpatient clinics are now being established to better characterise cSVD, screen for modifiable risk factors, identify associated clinical syndromes and adopt a holistic approach to reviewing patients.

The management of large vessel stroke has benefitted from significant recent advancements in management and is now largely algorithmic in the acute setting, dependent upon essential parameters such as time from onset in the case of thrombolysis and thrombectomy. This is followed by secondary prevention measures such as hypertension and lipid management, lifestyle interventions (particularly smoking cessation) and long-term antiplatelet therapy. Several guidelines are available summarising the most up to date recommendations.[54-56] However, management options for lacunar stroke are limited and it is notable that many recent stroke interventional studies did not specifically examine efficacy in lacunar syndromes.[57]

Management guidelines are also available to guide assessment and management of vascular cognitive impairment - though there is currently insufficient evidence to support the use of cholinesterase inhibitors which are routinely recommended in other dementia syndromes such as Alzheimer's disease.[58] The rates, risks and routes to reduce vascular dementia (R4Vad) study is a multi-centre observational cohort study of cognition after stroke which may provide additional future insights into this population.[59]

For covert cSVD, the recent European Stroke Organisation (ESO) guideline for the management of covert cSVD provides expert consensus statements on current evidence, centred around promoting a healthy lifestyle with regular exercise, smoking cessation, diabetes and hypertension management.[60] Theoretically, early identification and management could reduce the burden of advancing cSVD. However, there is currently a lack of direct prophylactic and therapeutic strategies (i.e. disease modifying therapy) available.[28] This is partly due to our incomplete understanding regarding the pathophysiology of cSVD – particularly given the heterogeneity of conditions falling under this umbrella – and the inherent difficulties of recruiting patients with covert cSVD to clinical studies. By the time that cSVD manifests clinically, or even to a significant extent covertly upon conventional imaging, cSVD may be less amenable to disease modifying therapy.

Nevertheless, several repurposed treatments are being evaluated including phosphodiesterase-5 inhibitors, glucagon like peptide-1 analogues and allopurinol, but these have yet to show clear benefits.[61] The Lacunar Intervention Trial-2 (LACI-2) demonstrated that the use of isosorbide mononitrate and cilostazol were well tolerated and safe for use in lacunar stroke patients and may reduce subsequent stroke, dependence and cognitive impairment but larger phase 3 trials are required.[62] There is currently no role for the use of antiplatelet therapies such as aspirin or clopidogrel in covert cSVD, particularly when considered against the small risk of associated side effects.[60]

It is clear that the development of future therapeutics depends upon a greater understanding of cSVD pathophysiology and the validation of robust, non-invasive markers of cSVD on which to base clinical studies. Given the association between cSVD and BBB dysfunction, the ability to measure this process could be advantageous.

Blood-brain barrier dysfunction in cSVD:

Under normal conditions, the BBB separates the central nervous system (CNS) from the peripheral circulation to create a stable microenvironment whilst responding dynamically to both metabolic and immunological demands. The full phenotype of the BBB is bestowed upon the small brain capillaries and is notably absent within the circumventricular organs for the regulation of autonomic and endocrine function.[63]

The BBB (see Figure 1-3) is primarily composed of specialist brain-capillary endothelial cells (BECs). Compared to endothelial cells in the peripheral circulation, BECs have a flattened appearance, lack fenestrations and exhibit sparse pinocytic activity.[63] Transcellular transportation across the endothelium is highly selective, via either active or passive specialised transportation proteins.[64] BECs are bound together by tight junctions, made up of claudin, occludin and junctional adhesion molecules generating a high electrical resistance (~1500-2000 Ωcm^2) and limiting paracellular passage to small (<800 Daltons) lipophilic substances such as carbon dioxide and oxygen.[65-68]

The discovery of the *barrier* characteristic is often attributed to the work of Ehrlich and Goldmann, who demonstrated that injected large molecular weight dyes would not cross between the intravascular and CNS compartments – and vice versa.[69-72]

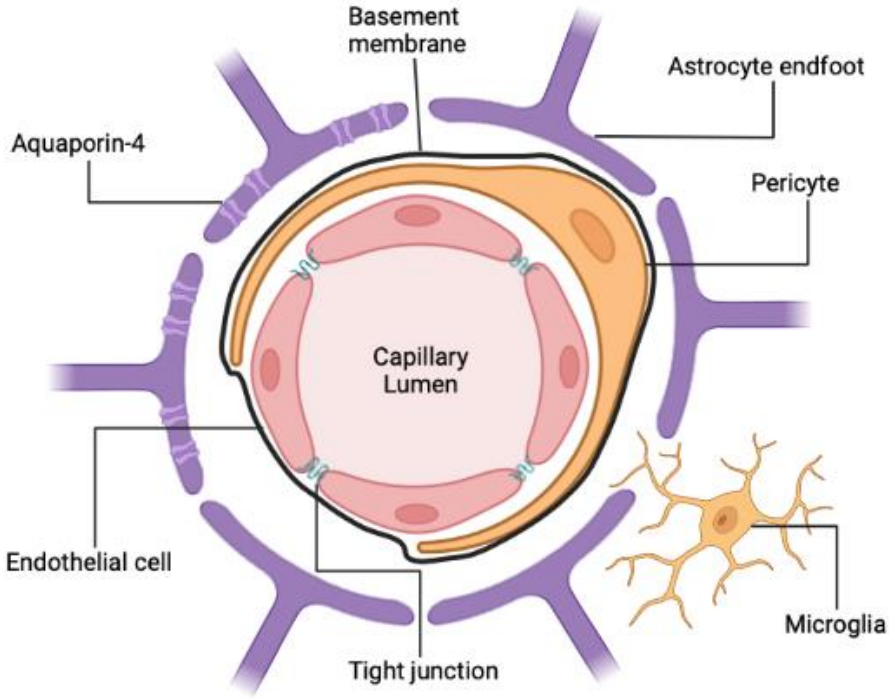


Figure 1-3: Simplified blood-brain barrier structure. Produced using Biorender.

Subsequent electron microscopy (see Figure 1-4) has shown the close proximity of astrocytes and pericytes to BECs, with astrocyte perivascular endfeet overlapping and interdigitating to ensheath approximately 90% of the BEC surface area.[73] Astrocytes are the most abundant glial cell in the CNS and are involved with maintaining water, ion and glutamate homeostasis, angiogenesis, neurogenesis and synapse modulation.[74] Pericytes cover around 30% of the surface area of BECs and share a common basement membrane.[75,76] Pericytes appear to regulate blood flow and immune cell entry to the CNS, alongside essential roles in blood vessel formation.[76,77]

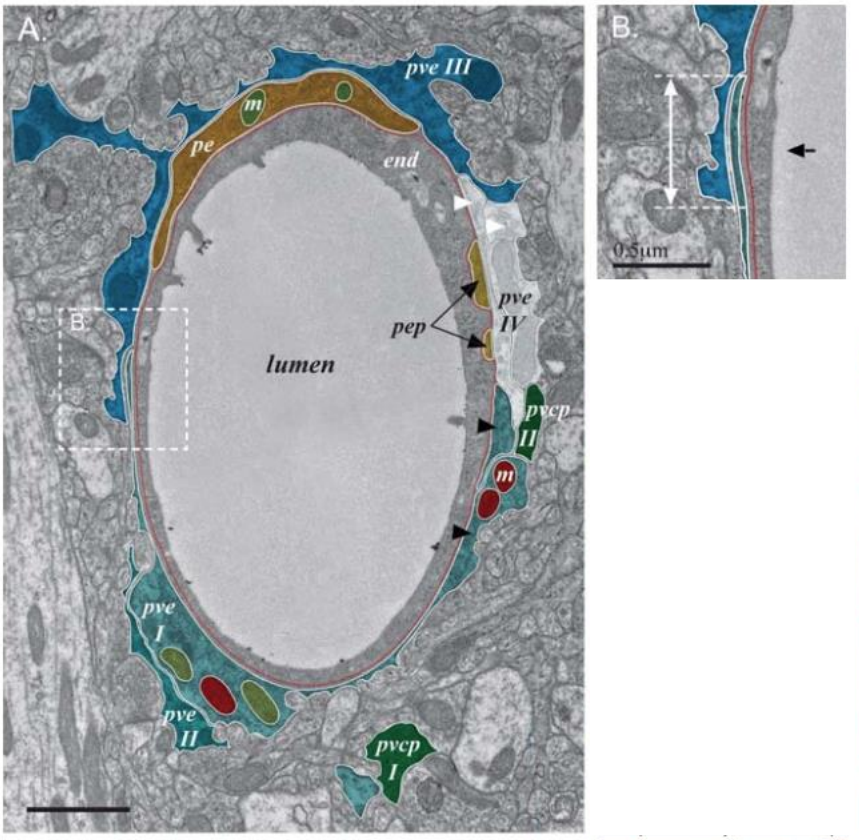


Figure 1-4: Electron microscopy of the BBB, taken from Mathissen *et al*.[73] (A) Electron microscopic image of a capillary with four endfoot profiles (*pve I-IV*). The hatched area (enlarged in B) shows the endfoot-endfoot overlap. The double arrow in B represents the projection of the overlap.

Often, the term BBB is actually used to refer to the wider neurovascular unit, including BECs and the surrounding neurones, astrocytes, pericytes, microglia, extracellular matrix and basement membrane.[78] Indeed, a neurone is never farther than 10-20 micrometres from the nearest capillary.[79] The complex interplay between these constituents is incompletely understood, but direct interaction facilitated by their close proximity, generation of localised micro-gradients and induction of BBB properties through the release of chemical mediators such as cytokines appears essential.[80-84]

Although not directly related to this review, it is worthwhile noting that several other barriers exist within the CNS. These include the blood-cerebrospinal fluid (blood-CSF) barrier at the highly vascularised choroid plexus (responsible for the production of CSF), the ependymal cells lining the cerebral ventricular walls and the physical meninges (dura, arachnoid and pia mater) which form the outermost protective layer of the CNS.[85,86]

Research is ongoing to fully elucidate the underlying mechanisms, but BBB dysfunction refers to a heterogeneous process involving alteration to any part of the structure and/or functioning of the BBB (or likely the neurovascular unit) with downstream consequences such as oedema, neuroinflammation and neurodegeneration.[87-89] This process is likely to differ according to ageing, pathology, acuity and previous insults such as environmental exposures and perhaps even the gut microbiota.[90] For example, reduced tight junction integrity results in extravasation of blood products including toxins, plasma proteins and immune constituents causing microglial activation.[91] However, conversely glial cell modulation of tight junctions may be involved with initiation or propagation of this process.[92] BBB dysfunction has now been implicated in normal ageing as well as a range of pathologies (see Table 1-5) and remains a core mechanism in the pathophysiology of cSVD.[93,94]

For example, in the case of acute ischaemic stroke, soon after vessel occlusion and hypoperfusion occurs ischaemia triggers an inflammatory response with excess production of reactive oxygen species (ROS), increase of matrix metalloproteinases (MMPs), infiltration of immune cells (neutrophils, monocytes and T-lymphocytes) and activation of microglial cells, perpetuated by the release of proinflammatory cytokines.[95,96] MMPs degrade the tight junctions between BECs, increasing paracellular permeability and extravasation of blood products.[96] These factors have been shown to impair BBB function and increase permeability, impede

neuronal activity and play a critical role in the potentially devastating sequalae of haemorrhagic transformation.[97,98]

Neurodegenerative:
Alzheimer's Disease[99,100]
Parkinson's Disease[101]
Neuroinflammatory:
Multiple Sclerosis[102-104]
Neurovascular:
Ischaemic / Haemorrhagic stroke[105]
Subarachnoid haemorrhage[106]
Cerebral small vessel disease[1,29,107]
Reversible cerebral vasoconstriction syndrome[108]
Neurooncology:
Primary brain malignancy (Glioma)[109]
Brain metastases[110]
Neurological:
Epilepsy[111,112]
Traumatic brain injury[113-115]
Autoimmune / Connective Tissue Disorders:
Systemic Lupus Erythematosus[116-119]
Infection:
Meningoencephalitis[120]
Neuroborreliosis[121]
HIV[122]
Wider pathologies:
Following cardiopulmonary bypass[123]
Sickle cell disease[124]
Type 2 diabetes mellitus[125]
Pre-eclampsia[126]
Systemic malignancy – e.g. Lung cancer[127]
Normal ageing[128]

Table 1-5: Blood-brain barrier dysfunction is implicated in a range of pathologies.

BBB dysfunction in cSVD appears to be a more diffuse process.[129] In an animal model of cSVD, using spontaneously hypertensive stroke-prone rats, widespread BBB dysfunction was

noted with endothelial injury, erythrocyte stasis and altered vessel architecture.[130] Human radiological studies have shown increasing WMH burden is associated with greater BBB dysfunction,[131] with leakage of contrast agents seen within WMHs as well as within the normal appearing grey and white matter when compared to healthy controls.[33] BBB dysfunction is also associated with other radiological markers such as enlarged perivascular spaces and cerebral microbleeds.[107,132,133] In a 2-year longitudinal study of patients with vascular cognitive impairment, increased BBB dysfunction was associated with a higher rate of cognitive decline.[93]

There are currently several unanswered questions regarding BBB dysfunction in cSVD, including how this relates to compensatory or wider brain homeostatic mechanisms such as the recently described glymphatic system.[134,135] Further research is also required to understand how to differentiate this process from normal ageing.[136] However, being able to measure BBB function may assist with earlier diagnosis and treatment, with several potential therapeutic targets already considered.[137]

Measuring BBB dysfunction:

Current methods of assessing BBB function include analysis of serum samples for the presence of brain derived proteins, measuring protein levels in cerebrospinal fluid (CSF) and specialised radiological studies.

Levels of several brain derived proteins within the peripheral circulation, such as neuron-specific enolase, glial fibrillary acidic protein and S-100β have been investigated.[138,139] For example the calcium binding protein S-100β, a protein involved with maintenance of cell homeostasis and cell-cell communication mainly produced within astrocyte endfeet, has been

shown to be released into the peripheral circulation when the BBB is disrupted.[140-143] When compared to the general population, S-100β is significantly higher in patients presenting with acute middle cerebral artery infarction with severe neurological deficits at the time of admission.[144] However, whilst only requiring a simple blood test, the specificity of S-100β is limited as the molecule can be released from elsewhere, baseline circulating levels of S-100β vary between individuals and when S-100β is elevated there is no topographical information available regarding the site of BBB dysfunction.[145]

Analysis of CSF, obtained via lumbar puncture (LP), to measure constituents such as cell count and protein (predominantly albumin) is part of the routinely completed repertoire of diagnostic tests in neurological disease. Under normal conditions, albumin levels within the CNS are low. In the context of BBB damage, reduced tight junction integrity results in extravasation of protein from blood plasma, with comparison of CSF/serum albumin levels to calculate an albumin index (or *quotient*) providing a quantitative measure of BBB dysfunction.[146-148] Despite several limitations, this is well documented to be elevated in conditions such as multiple sclerosis.[149] However, this measure is reliant upon paracellular protein leakage between BECs and does not reflect the wider aspects of the neurovascular unit, does not provide topographic information regarding the site of BBB dysfunction, nor does it account for the known CSF constituent changes seen with normal ageing or concurrent pathology such as diabetes mellitus - whereby the CSF protein is widely reported to be elevated.[150] Further, albumin is a relatively large molecule and whilst elevated CSF albumin levels have been associated with cSVD,[151] a critical threshold of tight junction dysfunction would be expected prior to leakage across the BBB, potentially limiting sensitivity in early disease.[152] Performing an LP is safe, but is also relatively more invasive than blood sampling (potentially limiting longitudinal study) and can be associated with complications such as headache/CSF leak.[153]

LP may also be contraindicated in the context of anticoagulation or raised intracranial pressure.[153,154] Recently, alternate CSF markers of BBB dysfunction such as elevated levels of platelet-derived growth factor-beta have been proposed and investigation is ongoing.[155]

Radiological studies can potentially obtain non-invasive, high-resolution topographical analysis of BBB dysfunction. Nuclear imaging, with either positron emission tomography (PET) or single photon emission computed tomography (SPECT), can provide a spatial and functional assessment of various BBB transporters.[156] For example, using the radionucleotide fluorodeoxyglucose (FDG) allows for the assessment of glucose metabolism.[157] By comparing regions of hypometabolism, PET can assist with differentiating patients with cognitive decline due to cSVD (in the form of vascular dementia) from those with other syndromes such as Alzheimer's disease.[158] PET can also assess for paracellular leakage of large molecules, reflecting tight junction integrity, with the use of gallium tracers.[96] However, cost, hardware requirements and the availability, half-life and patient exposure to radionucleotides limit their widespread application.[96]

MRI therefore remains the key modality for BBB imaging. In the clinical domain, MRI scanning pre- and post-injection of gadolinium-based contrast agents (GBCAs) in patients with gross BBB dysfunction such as stroke, active demyelination or high-grade gliomatous disease is well established. Under normal conditions, GBCAs do not cross the intact BBB due to their large molecular size. However, in the context of reduced tight junction integrity GBCAs extravasate into the surrounding tissues and, due to their paramagnetic properties, cause shortening of T1 relaxation times. This manifests as visually conspicuous hyperintense signal on T1- weighted (T1-w) imaging sequences.[159,160]

Dynamic contrast enhanced (DCE)-MRI represents the most established quantitative method of applying this technique to more subtle BBB dysfunction (Figure 1-5).[161-164] By obtaining multiple images over an interval period, the transfer of GBCA (K_{trans}) can be calculated. DCE-MRI has already shown widespread BBB leakage in cSVD, including within the normal appearing white matter.[33] Reproducibility studies of DCE-MRI have reported moderate to excellent results.[165]

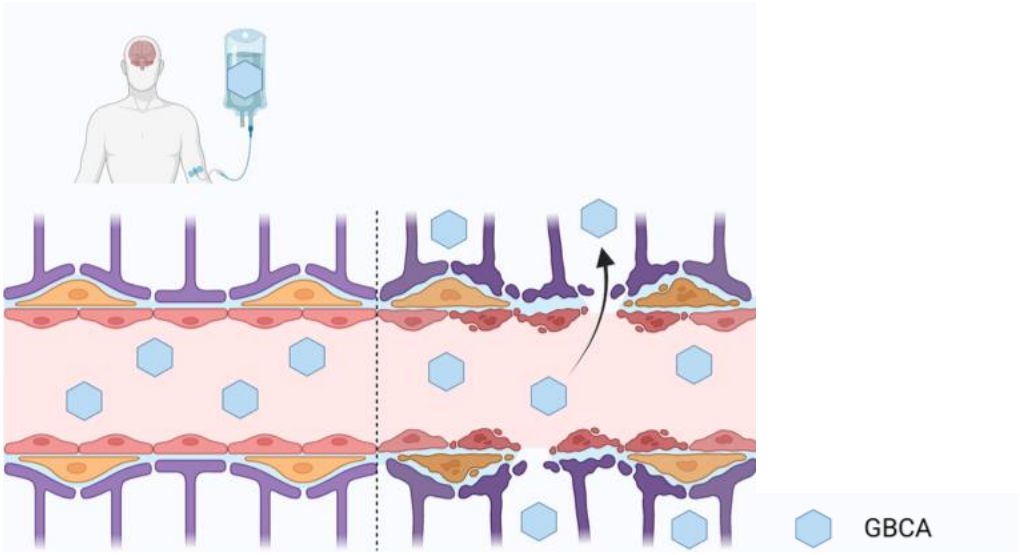


Figure 1-5: Graphical representation of DCE-MRI, adapted from Lee *et al*.[152] With an intact BBB, GBCAs are restricted from the brain parenchyma due to tight junctions binding brain endothelial cells together (left). GBCAs can extravasate into the brain via paracellular routes in the context of BBB dysfunction, which can be detected by DCE-MRI. (Creative Commons Attribution License – CC BY)

However, there are limitations to DCE-MRI. In gross BBB dysfunction, the post-contrast signal difference is a magnitude of 50-100% greater, whereas with subtle BBB dysfunction such as with cSVD this difference is <5%.[96] This can result in difficulty discriminating intra- and extravascular contrast agent and produce a lower signal to noise ratio (SNR) despite increased acquisition time.[166] Modelling assumes that any change in signal enhancement over a period of time is entirely due to contrast extravasation and attributes this simply to BBB

dysfunction, without considering factors such as scanner drift or the effects of BBB water exchange.[167] These factors may be negligible in the context of high flux GBCA extravasation, but are likely to be more important with subtle dysfunction. This results in relatively higher margins of error (necessitating large study sample sizes), with acquisition parameters and subsequent kinetic modelling assumptions also introducing systematic error.[168] Longitudinal study of DCE-MRI can be limited by the ongoing requirement for exogenous contrast agent administration, with the associated risk of hypersensitivity reactions, extravasation injury and potential for tissue gadolinium deposition (particularly in the context of impaired renal function which is prevalent in populations such as those with cSVD).[169-172]

Dynamic susceptibility contrast MRI, measuring T2-weighted or T2*-weighted signal changes to evaluate brain perfusion properties has been trialled but is not currently recommended in subtle or low-flux BBB dysfunction due to unfavourable error margins.[1,159]

Acknowledging these limitations, there is an ongoing shift towards developing contrast-free, MRI-based technologies to assess BBB function. Measuring water exchange across the BBB has recently been proposed as a promising solution.[173]

BBB water exchange:

Brain functioning is closely linked to water homeostasis, with water accounting for more than 90% of all molecules in the human body.[174] Water within the CNS is compartmentalised, present in either the blood, CSF, interstitial or intracellular fluid.[175] Complex osmoregulatory mechanisms protect the CNS from sudden, large shifts in water content secondary to external factors including fluctuations in cerebral perfusion pressure (hydrostatic pressure) and hydration (osmotic pressure).[176] When CNS water is acutely dysregulated, the potentially fatal

sequelae of cerebral oedema occurs – either due to accumulation of intracellular fluid (cytotoxic oedema) in the context of tissue ischaemia or interstitial fluid (vasogenic oedema) as a result of extravasation from BBB breakdown.[177,178]

At the BBB, water transportation is bi-directional, with net water flux likely to be a combination of simple osmotic diffusion (either transcellular or paracellular) alongside both active and passive transportation (see Figure 1-6). Consensus regarding exactly how this process occurs, and to what extent each component contributes to the overall movement of water, is yet to be reached. Research into this process is limited: altered gene expression profiles can be seen in cultured cell studies; purified capillary studies may still contain remnants of astrocyte endfeet and there are difficulties addressing the luminal/abluminal properties of the capillary endothelium.[179] However, an excellent recent review by MacAulay *et al.* has summarised our current understanding and dispelled many of the myths within this domain.[179]

In the peripheral circulation, water simply diffuses across the vascular endothelium in response to osmotic gradients.[176] Previously, this was also thought to be the case in the brain.[180] However, nuclear imaging studies have long shown that the first past extraction of labelled water is limited compared to freely diffusing lipophilic substances such as [^{11}C]butanol.[181] There is also high electrical resistance across the BBB, generated by the tight junctions between BECs, impeding the passage of ionic substances such as water..[175,182,183] Furthermore, focussed ultrasound disruption of the BBB in rats has been shown to modify local water transportation and when claudin-5 deficient mice (an essential tight junction component) are compared to wide-type, there is increased levels of paracellular leakage.[184,185] Movement across the BBB is therefore likely to be restricted.

Studies now estimate that the permeability of the BBB to water, in comparison to the vascular endothelium in the peripheral circulation, is at least 10-fold lower.[186] This is almost certainly an oversimplification, but given the large total surface area of the BBB even negligible levels of diffusion may contribute significantly to the overall flux of water.[180,186] Indeed, osmotically driven transport of water has been shown to occur in both animal studies and humans – with the intravenous administration of hyper-osmolar fluid to effectively draw water out of the brain via diffusion well-established in the management of conditions such as cerebral oedema.[187]

Aquaporins are specialised water transportation proteins, with aquaporin-4 the most abundant within the CNS and essential for water flux between brain fluid compartments including across the BBB.[174,180,188-190] Primary cell cultures of astrocytes taken from aquaporin-4 deficient mice demonstrate reduced osmotic water permeability when compared to wild-type specimens.[191] Brain oedema was shown to be significantly attenuated following acute water intoxication and ischaemic stroke in aquaporin-4 knockout mice.[192] In the longer term, aquaporin-4 suppression has been shown to result in compensatory increased cerebral vascularisation.[193] In humans, aquaporin-4 has been identified as an autoantigen in the neuroinflammatory condition neuromyelitis optica (NMO), with increased brain water content and larger infarct sizes in animal models of stroke when treated with NMO-IgG.[194]

However, when considering the role of aquaporin-4 specifically with relation to BBB water exchange this may be more complicated. Aquaporin-4 channels are almost, if not completely, absent from BECs and are instead present upon astrocyte endfeet - beyond the BEC basement membrane. Nevertheless, given the close physical proximity, aquaporin-4 may be involved with the production of osmotic gradients, the induction of regional adjustments to BBB

permeability via functional coupling with ion transporters or through the release of various astrocyte derived factors.[174,195,196] One such cytokine, interleukin-6 has shown a potentiating action on bradykinin mediated BBB opening.[196]

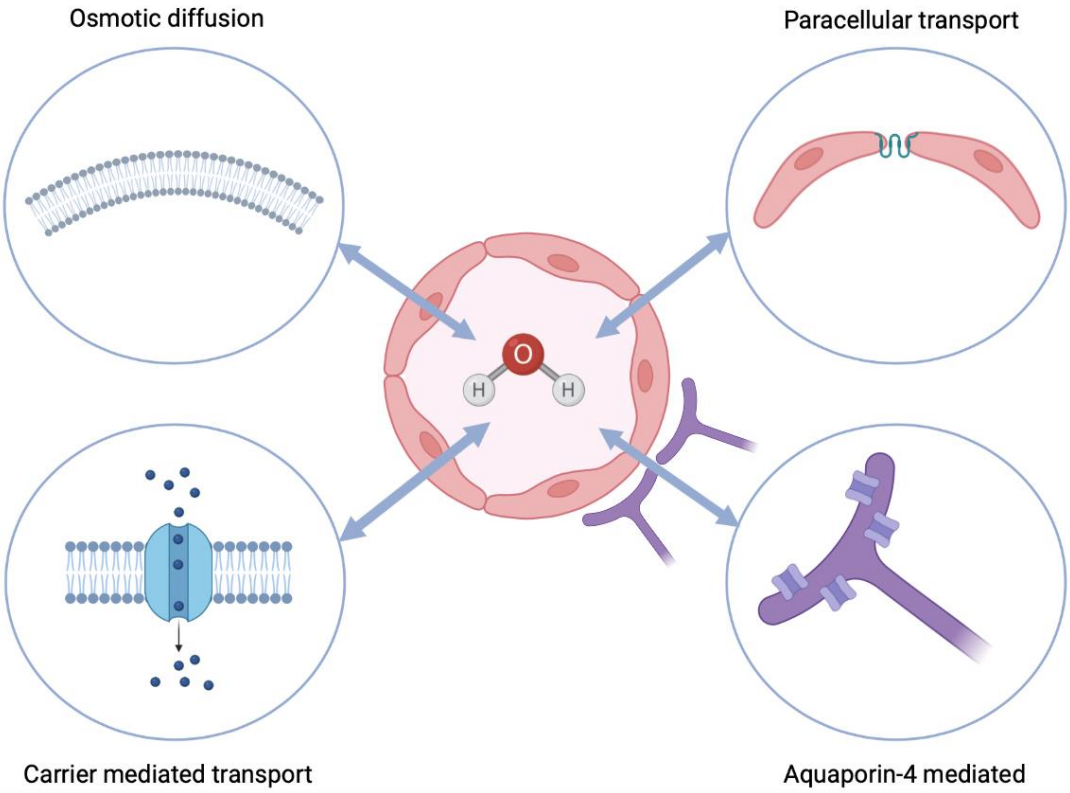


Figure 1-6: Overall BBB water exchange is likely to represent a complex interaction between osmotic diffusion, carrier mediated transport (active and passive) and paracellular transport/movement. In health, the presence of tight junctions restricts paracellular transport of water. Note that aquaporin-4 is located on astrocyte endfeet and whilst this has been shown to play an essential role in BBB water transportation it is located beyond the basement membrane. Created with Biorender.

Water can also be transported against an osmotic gradient, indicating that additional mechanisms alongside simple diffusion or passive facilitated transport must occur.[197] Several co-transporters have been identified, moving water alongside ions or essential molecules. Currently glucose (GLUT1), monocarboxylate (MCT1), glutamate (EAAT1) and Na^+/K^+-ATPase transporters have been reported, with some controversy regarding whether NKCC1 is

present within BECs.[174,179] Measuring water exchange may therefore reflect upon the metabolic function of the BBB.

How this process of water exchange differs in the context of BBB dysfunction is likely to be complicated by various factors such as age, pathology, acuity and co-morbidity. This could range from transiently reduced tight junction integrity to chronic BBB breakdown with altered enzymatic function and transport systems.[198,199] Fluctuations in water exchange could be due to changes in one, or more likely multiple processes including altered neurovascular coupling, altered functioning of the blood-CSF barrier at the choroid plexus and changes in the glymphatic system.[200] Animal gene knockout models only represent a single modality of dysfunction and are unlikely to account for downstream compensatory mechanisms or changes in alternate brain compartments. Indeed, the role of each BBB component may change function in disease. For example, one hypothesis suggests gaps between astrocyte endfeet may only become the rate limiting step of water flux in the context of significant paracellular passage of water (due to tight junction breakdown or aquaporin-4 channel impairment).[73,197,201]

Despite the complexity, water remains one of the most highly transferred substances across the BBB (alongside O_2, CO_2 and glucose)[180] and preclinical animal studies have shown measurable alterations in BBB water exchange in various pathologies.[202] Given the limitations of current methods of assessing for BBB dysfunction, this represents an area for further research.

Measuring BBB water exchange using MRI:

Measuring the process of BBB water exchange using MRI has been recently proposed.[173] When compared to measurements of GBCA leakage, water exchange techniques have been shown to

reveal BBB dysfunction earlier in the disease process, potentially offering a more sensitive measure.[203]

At present, there are three broad categories of MRI-based water exchange imaging, namely shutter-speed analysis of DCE-MRI, adaptations to arterial spin labelling (ASL) and the more recently proposed filter-exchange imaging (FEXI).

To our knowledge, techniques such as multi-flip angle multi-echo (MFAME) imaging[204] and water exchange index[205-207] have not translated from preclinical imaging of animal models into humans due to technical factors. Please refer to Dickie *et al.* for detailed information regarding each technique.[173]

Shutter-speed analysis of DCE-MRI:

DCE-MRI has been adapted to estimate water exchange using shutter-speed analysis (ss-DCE-MRI).[208] This method allows estimation of the transendothelial water exchange rate constant (K_{po}/K_{bo}) alongside the permeability surface area of water (PS_w) using a two compartment exchange model which incorporates the effects of finite water exchange on MR signal.

Whilst there have been some encouraging results in breast cancer data,[209] debate remains about modelling and interpretation of ss-DCE-MRI – even in noise free simulated DCE-MRI data – and this technique continues to be limited by the requirement for GBCA administration.[210]

Arterial spin labelling:

ASL is typically used to measure cerebral perfusion by 'labelling' intravascular water to act as an endogenous contrast agent.[211,212] Labelling is performed by inverting the longitudinal

magnetisation of protons in the blood, proximal to the imaging slice, and measuring a change in signal due to inflow of labelled blood into the tissue. By obtaining labelled images at a range of post-label delay times, along with a control image without labelling, the influx of labelled protons into the slice can be measured, with cerebral blood flow and arterial transit time estimated (Figure 1-7).[213]

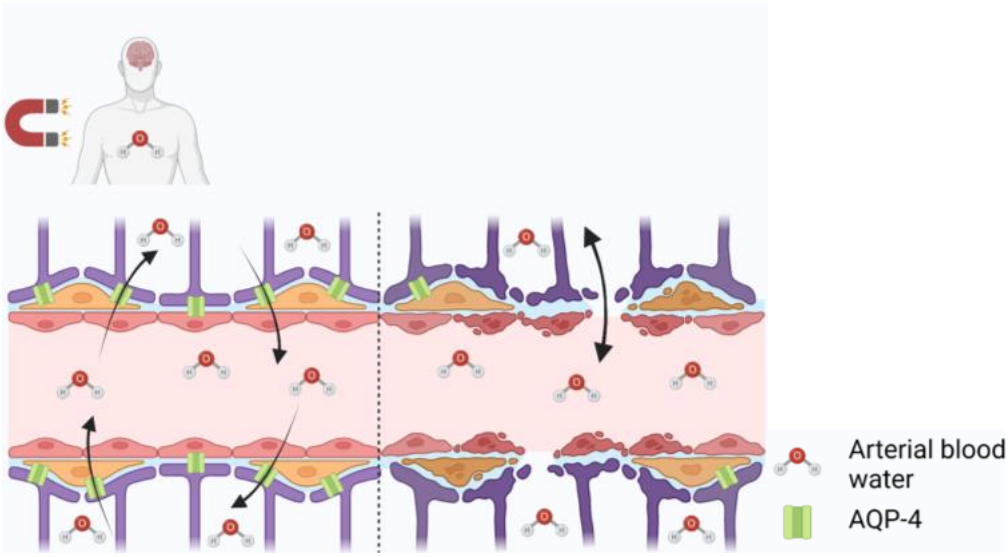


Figure 1-7: ASL MRI detects the movement of labelled water flux from the blood into the surrounding tissues (left). In BBB dysfunction, the mechanisms of water transport are altered (right). Adapted from Lee *et al*.[152] (Creative Commons Attribution License – CC BY)

This process can be further interpreted by two-compartment modelling to estimate the proportion of labelled water in the intravascular and extravascular space, allowing estimation of PS_w, K_{po} or the extraction of water (E_w).[212]

However, T1-w differences between intravascular and extravascular compartments are small, providing very low sensitivity to water exchange and a relatively low SNR. There are various strategies to improve SNR, such increasing scan times or administering GBCAs to enhance the difference between compartments.[214] Diffusion weighted ASL (DW-ASL) uses the pseudo-

diffusion coefficient of water molecules to differentiate tissues; approximately 100x larger in blood than in tissue.[215,216] Multiple echo time ASL (multi-TE ASL) uses T2-weighted (T2-w) imaging, taking account of the larger transverse relaxation time for blood water molecules than water within the brain.[217] Magnetisation transfer and phase contrast ASL have also been proposed.[218] No solution is without limitation, for example T2 signal can vary dependent upon blood oxygenation and DW-ASL relies on the assumption that diffusion gradients can fully remove vascular signal without impacting tissue signal. Further, pre-exchange capillary transit times are usually not included in the modelling but can be significant and may vary with disease.[219]

Focussed phase-contrast ASL methods have been developed to facilitate shorter acquisition times, namely Water Extraction with Phase Contrast Arterial Spin Tagging (WEPCAST).[220,221] Using WEPCAST, a whole brain estimation of water exchange is obtained by measuring the presence of labelled water draining through the superior sagittal sinus and inferring the amount of water extracted by brain tissue. With an estimate of CBF, a global value for PS_w can be calculated. This global measurement does not provide insight into regional BBB changes, but regional specificity may be gained by combining WEPCAST with vessel-encoding techniques.[222]

Filter-exchange imaging:

FEXI was originally designed to measure the rate of water exchange across cell membranes, but has recently been proposed as a method of measuring BBB water exchange imaging (also referred to as Vascular Exchange Imaging, or VEXI).[223] In principle, FEXI exploits the difference in diffusivity between intravascular and extravascular water (see Figure 1-8). Intravascular water has fast pseudo-diffusivity, in comparison to the slow diffusivity of

extravascular water. FEXI sequences are composed of two pulse gradient spin echo (PGSE) blocks separated by a longitudinal storage phase.[224,225] During the storage phase, water exchange occurs between the intra and extra vascular compartments and by varying this mixing time an ADC recovery curve can be measured. Fitting a simple one compartment model to the signal collected at a range of mixing times provides an estimation of the apparent exchange rate (AXR).

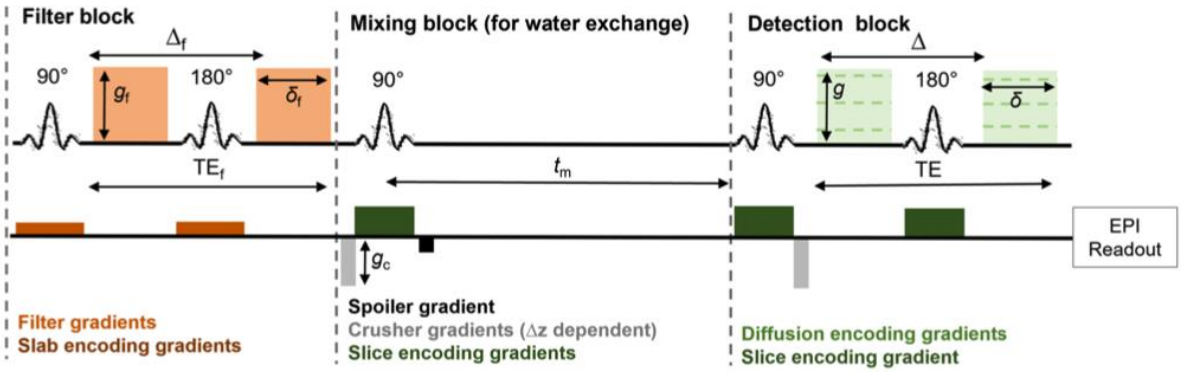


Figure 1-8: Fundamentals of FEXI. The filter block consists of a 90-degree excitation pulse with associated slab-selection (dark orange), followed by a fixed pair of diffusion-encoding gradients (with filter gradient amplitude (g_f), duration (δ_f) and diffusion time (Δ_f), light orange) separated by a slab selective refocusing pulse. The mixing block, for encoding water exchange during varying mixing time t_m, consists of crusher gradients (grey) (with crusher gradient amplitude (g_c) dependent on slice thickness (Δ_z)), slice encoding gradients (dark green) associated with the RF pulses, and a spoiler gradient (black) to null unwanted transverse magnetisation. The detection block, for signal readout, consists of variable diffusion encoding gradients (with gradient amplitude (g), duration (δ) and diffusion time (Δ), light green) followed by an echo planar imaging (EPI) readout. With permission from Ohene *et al.*[25]

Repeatability studies performed in healthy volunteers have recently demonstrated that FEXI offers a reliable approach to detecting subtle changes in BBB functioning.[226] However, there are several assumptions and simplifications in standard AXR modelling which may impact on the estimation of BBB water exchange. For example, assumption of equal T1 and T2 values in

blood and tissue, accounting for relaxation effects and the impact of 'crusher' gradients on diffusion weighting causing exchange rate-dependent bias in ADC recovery and underestimation of AXR.[226,227]

Conclusions:

In an ageing population and with finite healthcare resources, cSVD represents an evolving public health emergency. The diverse spectrum of cSVD, combined with the technical difficulties in visualising the small blood vessels, limit our current understanding of the condition. However, widespread changes in BBB functioning appear to be central to the underlying disease process.

Clinical manifestations of cSVD, prior to sequelae such as stroke or dementia, are often subtle and overlooked. When considering conventional radiological markers, such as WMHs, there is potentially little clinico-radiological correlation and indeed WMHs may be associated with several other pathologies. That said, the recently identified regression in WMHs in some individuals requires further evaluation and only serves to heighten the need for further understanding of cSVD pathogenesis.

Treatment options are at present limited to optimisation of cardiovascular health and modifiable risk factors, but the evidence to support these interventions in covert cSVD is limited. All studies of disease modifying therapies in cSVD have failed to reach significance so far, possibly because of the heterogeneous underlying pathology and lack of surrogate end markers.

Further understanding the BBB changes underpinning cSVD pathophysiology and the ability to detect this dysfunction with radiological studies such as BBB water exchange imaging may offer further understanding of the condition, earlier detection of the underlying white matter microstructural changes and the opportunity for meaningful intervention.[228] Advances are required to validate and standardise non-contrast BBB water exchange imaging techniques ideally utilising clinically available hardware and with acceptable acquisition times.

Chapter 2: Using routinely collected data to measure the local prevalence of cerebral small vessel disease.

Contribution statement:
I was directly involved with study conception and design. I was responsible for applying for service evaluation approval, data collection, analysis, interpretation and all parts of manuscript drafting and amendments. NR and JK provided statistical analysis support. SM was responsible for image analysis as part of the 2WW MDT.

Distribution:

Posters:

- **Maskery MP**, Mathur S, Knight J, Emsley HCA. Prevalence of radiological cerebral small vessel disease in the regional 2-week wait direct to scan MDT. Lancaster University Postgraduate Symposium (2023)

Poster tours:

- **Maskery MP**, Mathur S, Knight J, Emsley HCA. Prevalence of radiological cerebral small vessel disease in the regional 2-week wait direct to scan MDT. Association of British Neurologists Annual Meeting, Belfast (2023)

Publications:

- *(submitted: in peer review)* **Maskery MP**, Rennie N, Mathur S, Knight J, Emsley HCA. Prevalence of radiological cerebral small vessel disease: an insight from routinely collected data. BMC Neurology.
- Available as a pre-print: https://www.researchsquare.com/article/rs-3625684/v1

Introduction:

Cerebral small vessel disease (cSVD) represents a highly prevalent spectrum of disorders resulting in significant healthcare burden worldwide.[3,61,229,230]

Often detected incidentally, radiological features of cSVD include white matter hyperintensities (WMHs) of presumed vascular origin, lacunes, prominent perivascular spaces, cerebral microbleeds and atrophy.[37] WMHs can also be associated with migraine, demyelination and traumatic brain injury.[48-50] Assessment of the spatial distribution and

morphology of WMHs can indicate their radiological aetiology, but when the burden of lesions is low they are often deemed non-specific.[19]

Clinically cSVD contributes to 20-25% of stroke and 45% of dementia, however a frequently overlooked prodrome of vascular parkinsonism, gait disturbance, falls, apathy, depression, incontinence and/or an emergent dysexecutive cognitive profile may be apparent upon clinical assessment.[231-233] cSVD is also implicated in the development of later life epilepsy.[13] When, or indeed whether, these clinical phenotypes materialise may be associated with individual physiological reserve, compensatory mechanisms or may represent the heterogeneous underlying pathophysiology.[11,28] Rather than an ubiquitous component of the normal aging process, it is clear that radiological evidence of cSVD bestows adverse physical and prognostic implications.[34,234,235]

The management of incidentally discovered cSVD, evident on imaging performed for alternate reasons, is largely summarised by the recent ESO guideline.[60] The mainstay of therapy comprises simple holistic measures such as lifestyle modification and hypertension management.[11] Several studies support active surveillance and management of hypertension in cSVD, with a target systolic blood pressure of <130mmHg associated with reduced WMH progression.[236-238] Otherwise, there is no specific disease modifying therapy for cSVD and antiplatelet therapy is not recommended unless indicated for other co-existent factors such as previous stroke or transient ischaemic attack, nor do recommendations directly address covert cSVD in younger populations.[57,60]

Addressing this significant public health concern is vital, particularly with an ageing population. Prospective population studies have already demonstrated the high prevalence of

WMHs in this elderly cohort, but with increasing availability of electronic patient records and approximately 900,000 MRI brain scans performed in the United Kingdom annually, there is considerable scope for routinely collected data to improve our understanding of cSVD prevalence, demographics and risk factors throughout the wider population.[40,239,240]

Methods:

We identified our local two week wait (2WW) referral pathway for suspected central nervous system (CNS) cancer as a potential data source for further analysis. For this Lancashire specific referral stream, patients meeting certain referral criteria, including recent onset headache, rapidly progressive or subacute focal neurological deficit and/or other symptoms attributable to the central nervous system (see Table 2-1) are routinely invited for an expedited direct-to-scan MRI brain appointment followed by discussion in a mini-multidisciplinary team meeting attended by a consultant neurologist with a specialist interest in cerebrovascular disease and a consultant neuroradiologist. Considering the clinical information available on the structured referral proforma in the context of the radiological findings, there is direct feedback to the referring clinician to guide ongoing care. Previous studies of this referral stream have shown that the majority of patients (~97%) do not have an underlying malignancy.[241]

2WW mini-MDT referral criteria:
Progressive neurological deficit
Headaches of recent onset
Posture related headache
Vomiting
Drowsiness
Pulse-synchronous tinnitus
Papilloedema
Unilateral sensorineural deafness
Personality changes – for which there is no reasonable explanation
New/recent onset seizures
Unexplained cognitive impairments/behavioural disturbance/slowness
Cranial nerve palsy

Table 2-1: Referral criteria for the local 2WW suspected CNS cancer mini-MDT.

We retrospectively reviewed patient demographics, structured referral documentation and imaging outcomes between June 2020 and January 2023. This included patient's age, sex, World Health Organisation (WHO) performance status classification (see Table 2-2), 2WW referral criteria, imaging modality and findings. For each scan, the attending consultant neurologist and neuroradiologist routinely evaluated for the presence of WMHs, their suspected radiological aetiology (non-specific, equivocal, vascular or demyelinating) and a subjective measure of radiological burden. WMH burden was measured between 0 – 5 on a visual rating scale (0 – nil present, 1 – mild/few, 2 – mild to moderate, 3 – moderate, 4 – moderate to severe, 5 – severe/extensive).

0	Able to carry out all normal activity without restriction
1	Restricted in strenuous activity but ambulatory and able to carry out light work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Symptomatic and in a chair or in bed for greater than 50% of the day but not bedridden
4	Completely disabled; cannot carry out any self-care; totally confined to bed or chair

Table 2-2: World Health Organisation (WHO) performance status classification

For the initial analysis, all WMHs were included to assess their overall prevalence, with subsequent delineation into groups deemed 'cSVD' (WMHs of presumed vascular aetiology, grade 1 – 5) vs 'no cSVD' (no WMHs or WMHs deemed non-specific/equivocal or demyelinating).

Postcode data was cross-referenced with Lower-Layer Super Output Areas (LSOAs) to obtain 2019 UK government Open Data deciles for measures including the overall Index of Multiple Deprivation (IMD), Education, Skills and Training Deprivation (EST) and Health Deprivation and Disability (HDD).[242]

Chi-squared tests were used to compare findings between groups and logistic regression models were fitted to assess the relationship between cSVD, patient demographics and referral criteria.

This study was based entirely on retrospective analysis of routinely collected data and was deemed to represent a service evaluation registered at Lancashire Teaching Hospitals NHS

Foundation Trust. Ethics approval was therefore not required. Data was analysed using R version 4.1.3.[243]

Results:

Population demographics:

We retrospectively identified a total of 1058 mini-MDT records from this 30-month period. We excluded 25 records due to incomplete or duplicated data. Of the 1033 records included, the mean age was 51.3 ± 18.3 years with females representing 65% of patients.

Over 99% of patients underwent an MRI brain, with 4 subjects alternatively undergoing a CT brain due to severe claustrophobia, contraindications (e.g., permanent pacemaker) or patient preference.

As expected, many proformas had multiple 2WW referral criteria selected. Headache and symptoms raising suspicion of raised intracranial pressure represented the most frequent concern (headaches of recent onset n=662, posture related headache n=333, vomiting n=138, pulsatile tinnitus n=58 and papilloedema n=10).

Prevalence of WMHs and cSVD:

Overall, 451 (43.7%) scans demonstrated evidence of WMHs with 60% of these scans deemed in keeping with cSVD (see Table 2-3). The prevalence of WMHs increased with age, from approximately 20% of patients under 50 years old to almost 90% of those over 80 years (see Figure 2-1a). Advancing age also appeared to correlate with increasing radiological burden of WMHs (see Figure 2-1b).

	Overall	Males	Females
WMHs by age group			
<50 (%)	82 / 472 (17.4)	24 / 142 (16.9)	58 / 330 (17.6)
50 – 59 (%)	95 / 185 (51.4)	36 / 71 (50.7)	59 / 114 (51.8)
60 – 69 (%)	123 / 186 (66.1)	46 / 69 (66.7)	77 / 117 (65.8)
70 – 79 (%)	99 / 132 (75.0)	35 / 55 (63.7)	64 / 77 (83.1)
>80 (%)	52 / 58 (89.7)	23 / 25 (92.0)	29 / 33 (87.9)
WMHs by aetiology			
Non-specific (%)	174 (38.6)	63 (38.4)	111 (38.7)
Demyelinating (%)	9 (2.0)	4 (2.4)	5 (1.7)
Vascular (%)	268 (59.4)	97 (59.1)	171 (59.6)
WMHs by age group and aetiology			
	Non-specific	**Vascular**	**Demyelinating**
<50 (%)	71 / 82 (86.6)	6 / 82 (7.3)	5 / 82 (6.1)
50 – 59 (%)	56 / 95 (59.0)	37 / 95 (38.9)	2 / 95 (2.1)
60 – 69 (%)	36 / 123 (29.3)	85 / 123 (69.1)	2 / 123 (1.6)
70 – 79 (%)	10 / 99 (10.1)	89 / 99 (89.9)	0 / 99 (0)
>80 (%)	1 / 52 (1.9)	51 / 52 (98.1)	0 / 52 (0)

Table 2-3: Population demographics and prevalence of White Matter Hyperintensities (WMHs) by age group and radiological aetiology.

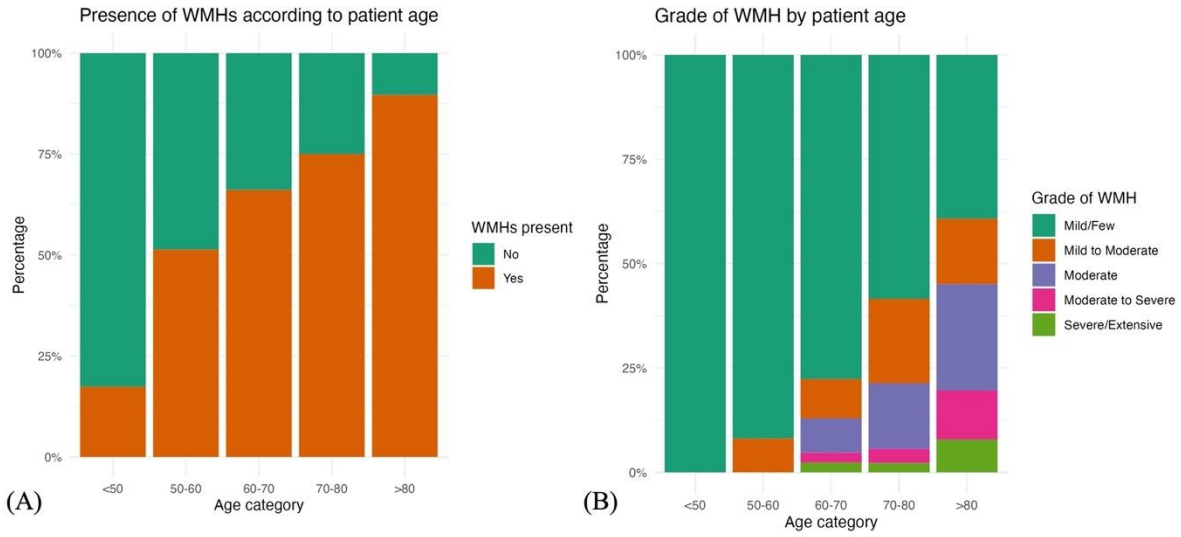


Figure 2-1: (A) Prevalence of White Matter Hyperintensities (WMHs) according to patient age group and (B) WMH burden according to patient age group.

Age also appeared to influence the radiological aetiology of WMHs (see Figure 2-2). Under the age of 50, WMHs were considered non-specific in 86% of scans where they were present, compared to <30% in those over 50. Indeed, after age 50, the majority of WMHs were attributed to cSVD, rising to >90% of cases in patients over 70 years old.

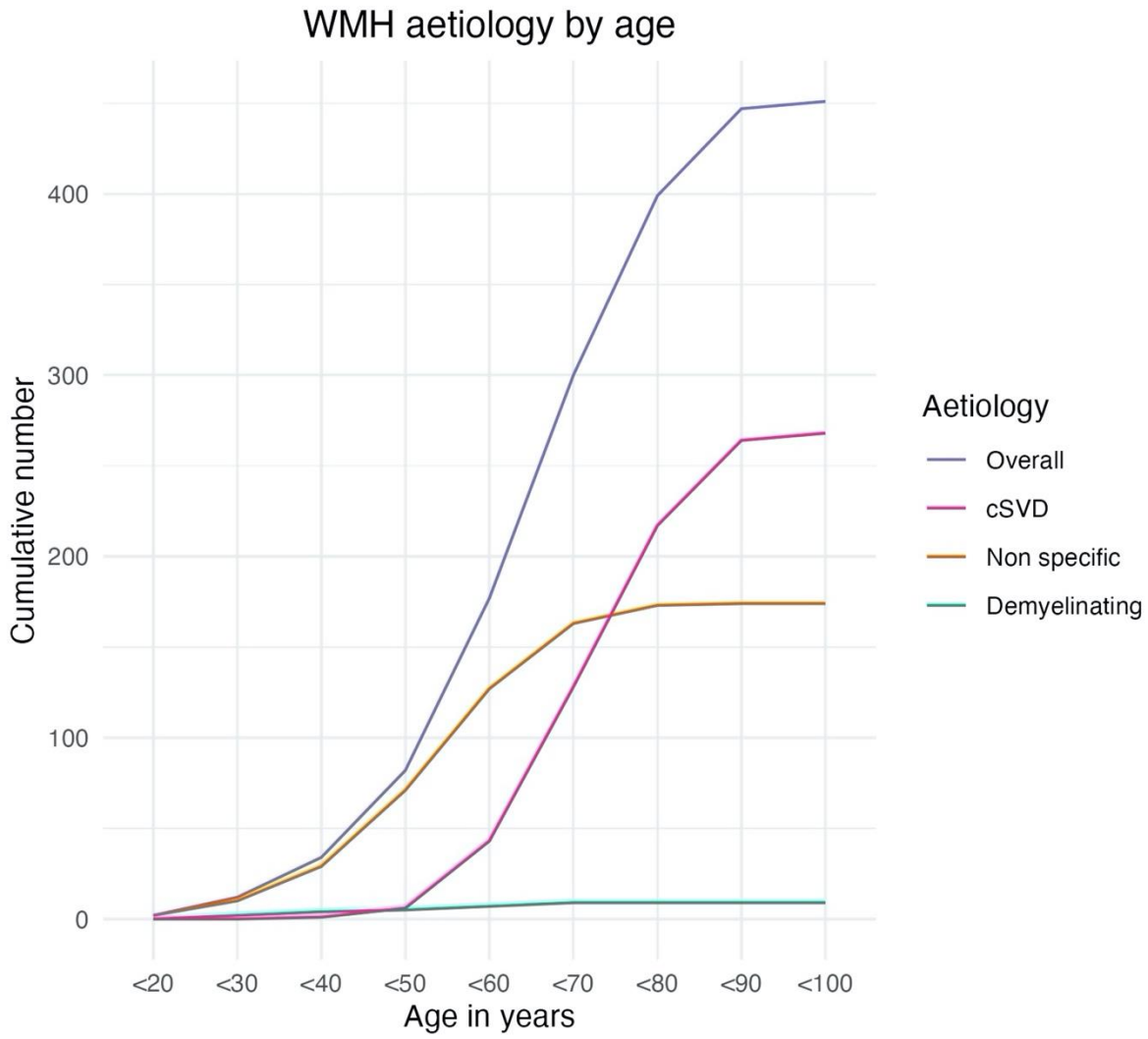


Figure 2-2: Aetiology of White Matter Hyperintensities (WMHs) by age group.

There was no significant difference in the presence of overall WMHs (p=0.47) or when specifically deemed indicative of cSVD (p=0.70) between males and females.

The proportion of patients with cSVD appeared higher in participants with WHO performance score ≥1 (see Figure 2-3), indicating a degree of restricted activities. This was confirmed by Chi-squared test (p<0.001).

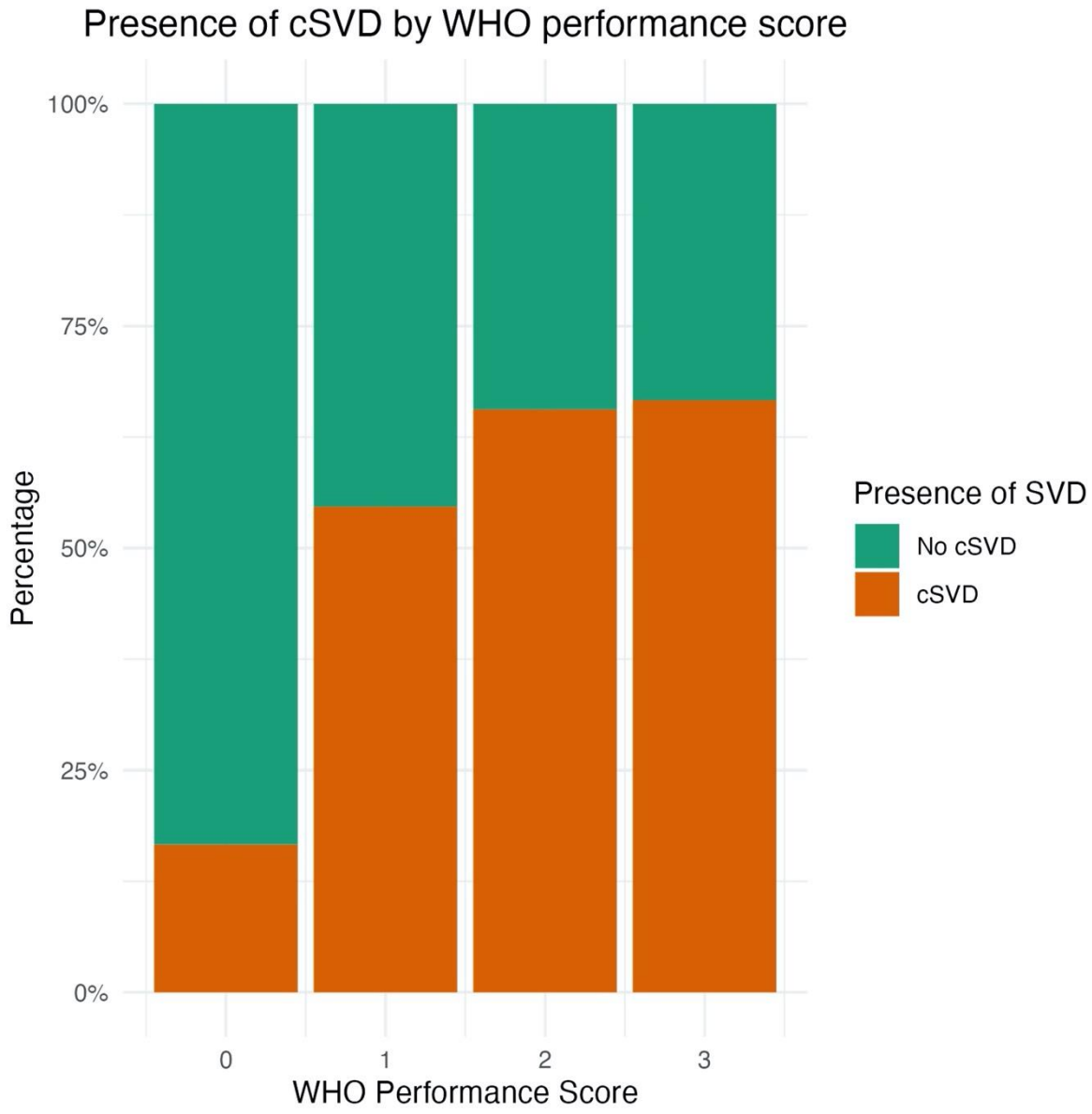


Figure 2-3: World Health Organisation (WHO) performance score according to presence of cerebral small vessel disease (cSVD)

Risk factors for cSVD:

We performed logistic regression modelling to investigate which patient demographic factors impacted the likelihood of identifying cSVD. We found a significant relationship between age and presence of cSVD with a 14.5% increase in the odds ratio with each increased year of age (p<0.001). When we performed a sensitivity analysis, whereby 10% of the radiological cSVD diagnoses were randomly inverted, age remained significant (p<0.001). Higher EST decile (indicative of higher levels of deprivation in terms of education, skills and training) was also associated with an increased risk of cSVD (p<0.05). Conversely, higher IMD decile was associated with a reduced risk of cSVD (p<0.05). HDD decile was non-significant (p=0.36). Patient sex (p=0.07) and WHO performance score (p=0.55) also did not reach statistical significance. Considering the magnitude of impact age had upon cSVD, we repeated a second model to only account for this factor. Whilst age remained a significant factor (p<0.001), AIC increased indicating that the additional demographic factors improved model fitting.

We also performed logistic regression modelling to identify whether any of the referral criteria were associated with cSVD. This revealed 'headaches of recent onset' (p<0.001) and 'drowsiness' (p<0.05) were associated with reduced cSVD prevalence. This was not entirely unexpected, as sleep disorders are well documented in cSVD.[244] However upon further inspection, it appeared these criteria were more common in younger patients and performing a conditional regression analysis adjusting for age reduced the significance of these findings.

Whilst the structured referral proforma does include designated fields for the recording of vascular risk factors and previous stroke, these were inconsistently documented and therefore excluded from our analysis.

Additional imaging findings:

In addition to WMHs, radiological abnormalities were identified on 438 (42.4%) scans (see Table 2-4). These were most commonly either ear, nose and throat (ENT) abnormalities (e.g., sinus thickening), soft radiological signs of raised intracranial pressure (e.g., partially empty sella turcica, optic nerve tortuosity, enlarged optic nerve sheath or flattening of the optic nerve head) or vascular abnormalities (e.g., aneurysm, arteriovenous malformations or venous anomalies). Approximately 3% of scans demonstrated neoplastic disease (2.5% primary / 0.5% secondary brain tumours). Forty-five (4.4%) scans revealed >1 radiological abnormality.

Additional findings:	**N (% of 1033 scans)**
Recent infarct or haemorrhage	7 (0.7)
Neoplasm:	
Primary brain tumour	26 (2.5)
Of which are meningioma	18 (1.7)
Metastatic brain tumour	5 (0.5)
Cerebellar descent / tonsillar ectopia / Chiari malformation	38 (3.7)
ENT abnormality (e.g., sinus thickening)	81 (7.8)
Developmental brain anomaly	9 (0.9)
Pituitary signal change / suspected adenoma	19 (1.8)
Soft radiological signs of raised intracranial pressure	89 (8.6)
Microhaemorrhages or suggestive of cerebral amyloid angiopathy	11 (1.1)
Radiological signs of normal pressure hydrocephalus	7 (0.7)
Non-specific gliosis	44 (4.3)
Arachnoid cyst	11 (1.1)
Pineal cyst	18 (1.7)
Aneurysm / venous anomaly / AVM	46 (4.5)
Bone lesion	13 (1.3)

Table 2-4: Additional radiological findings on routine 2WW brain imaging. Abbreviations: ENT: Ear, Nose & Throat, AVM: Arteriovenous Malformation.

Discussion:

Using routinely collected data we demonstrate a high prevalence of WMHs and cSVD in the elderly population, comparable to prospective cohort studies and the wider literature.[40] We also emphasise that WMHs are prevalent amongst the younger population.

In younger patients, WMHs are frequently deemed non-specific. Based on our findings, it is conceivable that many of these scans are actually indicative of mild cSVD. This may highlight a source of bias evident in routine neuroradiology reporting with reluctance to label changes as suggestive of cSVD in younger age categories combined with the limitations of current imaging techniques. These patients may benefit from simple, targeted interventions to mitigate vascular risk such as screening for hypertension, smoking cessation and advocating regular exercise. Similarly, whilst rigorous blood pressure targets may not be suitable for all elderly patients who may be prone to postural hypotension and falls, this may be more suitable in this lower age group.

Communicating the importance of low burden covert cSVD to seemingly asymptomatic patients can be challenging. However, WMHs on conventional imaging may reflect only the 'tip of the iceberg' when compared to the wider microstructural changes within normal appearing white matter.[17,245] Given the implications for subsequent stroke, dementia, neurodegeneration, physical dependency and death, earlier identification and discussion may serve as an opportunity for proactive intervention. Similarly, increased identification would facilitate recruitment to future cSVD research such as investigating genetic components, longitudinal WMH changes, novel imaging techniques to serve as surrogate trial endpoints and targeted therapeutics.[23,44,233]

We also found that cSVD is associated with performance score, indicating a degree of physical dependency or frailty in this group of patients. Further research is required to fully understand this finding, which may simply represent the typical ‘cSVD syndrome’. However, this does serve as a reminder of the potential benefits of preventing cSVD for preserving quality of life on a patient level and reducing the overall care burden on a population scale.

The association between LSOA-linked deciles of deprivation and cSVD was less clear, with increasing levels of health, skills and training associated with higher levels of cSVD with the converse true for IMD decile. Although interesting as a proof of concept, we acknowledge these findings may be artefact; a product of the relatively small geographical area covered by the local 2WW pathway and visualised by the vague correlations seen in Figure 2-4.

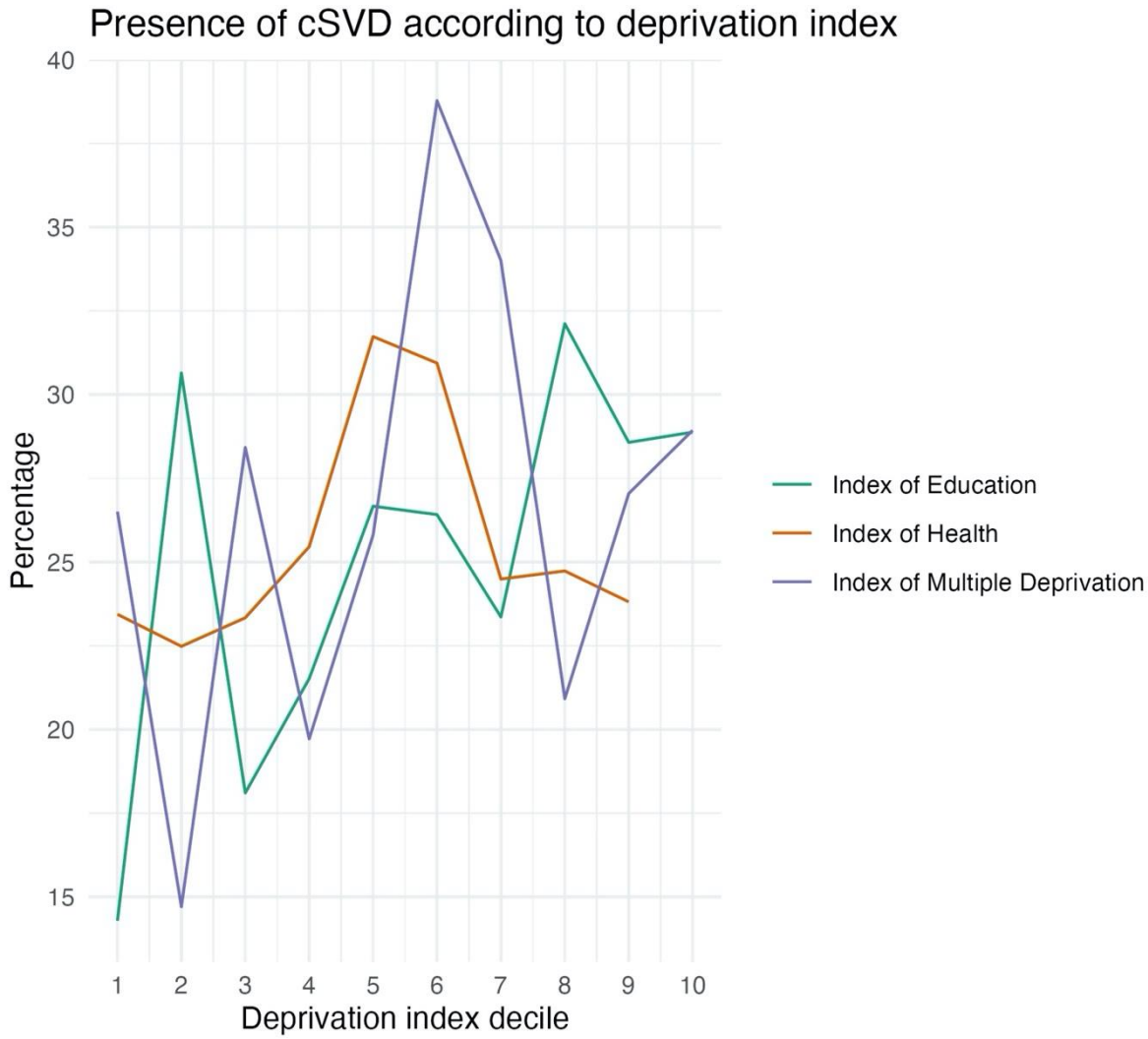


Figure 2-4: Deprivation index according to presence of cerebral small vessel disease (cSVD)

Similarly, the associations between cSVD and specific referral criteria should be interpreted with caution. Notwithstanding the often-pragmatic nature of referrals generated by routine clinical practice and the influence of age, this reflects the typical profile of patients referred via the 2WW CNS pathway with headache and the local referral architecture (e.g., suspected seizures are instead directed via the 'first fit' clinic). Nevertheless, larger studies across multiple referral streams and centres may improve this analysis. Developments in the

organisation of routinely collected data within healthcare systems should facilitate such analyses.

Given the deliberately selective nature of 2WW CNS referrals, we expected an increased number of additional imaging findings when compared with asymptomatic, prospective population studies.[246] The 3% rate of neoplastic disease is in line with suspected cancer referral criteria and comparable to a previous local study of this referral stream.[241] Nevertheless, we would argue that patients referred on this pathway (i.e. patients referred for imaging after seeking medical attention on the basis of being symptomatic) are representative of routine practice. With an increasing number of MRI scans performed each year, our findings vis a vis prevalence and nature of imaging findings may assist with counselling patients regarding the risk of incidental findings.

We acknowledge that this study does have limitations. Primarily, these are due to the variable granularity of routinely collected data, the single geographical area represented by referrals, the notional prerequisite for neurological symptoms suspicious for an underlying CNS malignancy and the unblinded analysis of imaging using a visual rating scale. Future analysis may benefit from a standardised approach to quantifying WMHs lesions such as the Fazekas score[247] with consideration of wider imaging markers of cSVD and commenting on their distribution. However, despite these factors we returned similar values to prospective studies with statistically significant outcomes.

The surprisingly high prevalence of WMHs in younger patients underlines the need to ensure careful clinical characterisation and further research to ensure that potential opportunities for intervention are not missed. While WMHs in the younger population are often deemed non-

specific, our findings are in keeping with at least a proportion being due to cSVD, so it is likely that enacting simple, standardised lifestyle interventions, including surveillance for hypertension, are reasonable in order to mitigate vascular risk. This study signals the potential for routinely collected data to further our understanding of cSVD prevalence, demographics, risk factors and clinical syndromes alongside facilitating targeted recruitment of well-phenotyped cSVD patients to future research studies.

Chapter 3: Reviewing the current MRI-based methods of measuring blood-brain barrier water exchange.

Contribution statement:
I was responsible for study conception and design, writing the review protocol and highly sensitive search strategy, screening of articles and drafting the manuscript. I performed all revisions to the manuscript based upon several rounds of feedback to produce the final manuscript for publication.

Distribution:
We intend to publish this as a manuscript in due course.

Introduction:

The blood-brain barrier (BBB) separates the central nervous system (CNS) from the peripheral circulation, creating a highly regulated microenvironment. Composed of a series of specialised brain endothelial cells (BECs), bound together by tight junctions and encapsulated by the surrounding astrocytes, pericytes, basement membrane, microglia and neurones the BBB has an essential role in maintaining a constant milieu to facilitate normal neurological function, dynamically facilitating the metabolic demands of the brain through processes such as neurovascular coupling and highly selective transportation, permits immunosurveillance and is notably absent in the circumventricular organs for the regulation of autonomic and endocrine function.[30,63]

Understanding of this complex interplay in health and disease is, as yet, incomplete.[248] Current clinical imaging modalities lack the resolution to directly visualise the smallest blood vessels and post-mortem studies are both limited in number and represent a static snapshot of late stage pathology.[26,27] *In vitro* studies allow for assessment of specific BBB characteristics but, similar to animal models, are naïve to the various complicating factors such as ageing, co-morbidity

and environmental exposure.[71,249] The BBB itself is also heterogeneous between brain regions, for example with areas such as the hippocampus more vulnerable to the effects of ageing and hypertension, it differs according to the respective level of the vascular tree and its function changes with the process of normal ageing.[128,248,250] Nevertheless, the role of gross BBB dysfunction in a range of acute neurological pathologies including infection and stroke is widely acknowledged.

There is now increasing evidence relating to subtle BBB dysfunction being fundamental in the early pathophysiology of a range of chronic neurodegenerative, neurovascular and neuroinflammatory conditions.[29,99-102] With the potential offer of improved diagnostics, disease prognostication and monitoring – notwithstanding the possibility of providing sensitive surrogate endpoints for future therapeutic studies – several techniques focussing on BBB dysfunction are now being investigated.

Whilst blood tests to measure the relative levels of brain-derived proteins in the peripheral circulation or comparison between blood and cerebrospinal fluid (CSF) albumin levels can provide a measure of BBB dysfunction, these lack specificity and are fundamentally unable to provide the topographical information inherent to imaging studies.[138,139,146,149] By altering the radionucleotide, nuclear medicine studies - either with Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT) - can probe specific BBB transporters, measure paracellular BBB leakage of large molecules such as gallium and provide a measure of cerebral metabolism.[156] However, concerns regarding overall scanning acquisition times as well as the availability, half-life, cost and patient exposure to radionucleotides and ionising radiation limits their application.[96]

Dynamic contrast-enhanced (DCE)-MRI is the most well-established method of measuring BBB dysfunction. Gadolinium-based contrast agents (GBCAs) are unable to leak across the intact BBB due to their large size. However, in the context of impaired tight junction integrity, GBCAs extravasate and cause a detectable signal change by shortening the T1 relaxation time of tissue.[167] By capturing a series of images, the contrast agent transfer constant (K_{trans}) can be estimated. DCE-MRI is most reliable where there is sizeable BBB dysfunction, when the post-contrast signal change is significant – however the signal to noise ratio (SNR) is less favourable in subtle, near normal or diffuse disease states, whereby it can be difficult to distinguish changes from background noise.[10,166,168] Concerns also remain regarding repeated exposure to GBCAs in longitudinal studies, particularly in the context of co-morbid renal disease.[96,166]

Measuring BBB water exchange using MRI has been proposed as promising alternative technique. Water is essential for normal neuronal functioning and is highly compartmentalised within the CNS – crossing the BBB via a combination of simple diffusion, active and passive transportation and influenced by aquaporin-4 channels located on astrocyte endfeet (see the excellent recent review by MacAulay *et al.* 2021 detailing our current understanding of this process).[176,179,181,251,252] Water exchange is thought to reflect processes wider than simple tight junction integrity, such as cerebral metabolism and changes in the glymphatic system.

Several methods of measuring BBB water exchange have been proposed (see Table 3-1 for parameters), predominantly centred around Arterial Spin Labelling (ASL), adaptations to DCE-MRI such as shutter-speed analysis (ss-DCE-MRI) and the recently described Filter Exchange Imaging (FEXI). The mechanics and technical aspects of each technique – alongside their limitations - have previously been described in reviews by Dickie *et al* and more recently by Elschot *et al.* and Harris *et al*.[159,173,253]

Briefly, ASL relies upon labelling water molecules (by applying a radiofrequency pulse to invert their spin) proximal to an imaging slice, then tracking the movement of labelled water at a series of post-label delay (PLD) times. A two-compartment model can then be utilised to estimate the proportion within the intra- and extra-vascular space to derive K_w (the BBB water exchange rate) and PS_w (the permeability surface area to water). Several methods have been studied to improve the SNR of ASL, such utilising contrast-enhanced (CE)-ASL protocols to increase the difference in T1-weighted (T1-w) relaxation times, T2-weighted (T2-w) imaging with multiple echo times (multi-TE)-ASL or exploiting the difference in diffusivity between compartments with diffusion weighted (DW)-ASL.

DCE-MRI can be adapted to measure BBB water exchange according to the difference in longitudinal relaxation rates between the intravascular and extravascular spaces induced by GBCAs.[254] Shutter speed analysis (ss-DCE-MRI) uses a two-compartment model to estimate the transendothelial water exchange rate constant (K_{po}/K_{bo}), PS_w and the contrast agent exchange rate constant (K_{pe}). However, there remains some controversy regarding interpretation of this modality.[210]

Filter exchange imaging (FEXI) exploits the difference in diffusivity between the 'fast' pseudo-diffusivity of water in the intravascular compartment and the slow diffusivity of water in tissue.[225] A filter is applied to effectively nullify the fast intravascular spins, followed by variable delay (or *mixing time*) during which water will naturally exchange between these compartments. By then repeating the imaging block, the Apparent Exchange Rate (AXR) of water can be calculated.

Parameter	Definition	Units
Arterial Spin Labelling (ASL) imaging:		
PS	Permeability surface area product – describes the exchange between compartments (e.g., PS_w – water)	ml water/min/100ml tissue[212] *or* ml/100g/min[255]
V	Volume fraction (e.g., V_b – blood, V_{bw} – blood water)	ml blood/ml tissue ml water/ml tissue
K_w / K_b	Exchange rate of water = PS/V_{bw}	min^{-1}
$K_{w\text{-}var}$	Kw variance (squared difference between ROI K_w and *normal* K_w)	$[min^{-1}]^2$
T_{exch} / T_{ex}	Exchange time for water across the BBB (see Mahroo *et al.*[256,257])	ms
K_{lin}	Tissue transition rate for water (see Mahroo *et al.*[257])	s^{-1}
E_w	Water extraction fraction	%
ATT	Arterial Transit Time	s
CBF	Cerebral blood flow	ml/min/100g
Dynamic Contrast Enhanced (DCE)-MRI (including shutter-speed imaging):		
K_{trans}	Volume transfer constant (or BBB leakage rate for GBCAs)	min^{-1}
K_{Gad}	Exchange rate of GBCAs $K_{Gad} = K_{trans} / V_p$	min^{-1}
K_{po}/K_{bo}	Water extravasation rate constant	s^{-1}
K_{pe}	Contrast agent molecule extravasation constant	s^{-1}
Filter Exchange Imaging:		
AXR	Apparent Exchange Rate	s^{-1}
K_{bo} / K	Vascular efflux constant	s^{-1}
b_f	Filter block	s/mm^2
t_m	Mixing time	ms

Table 3-1: Definitions and units of the main BBB imaging parameters

Given the considerable clinical need for improved diagnostics, disease monitoring and therapeutics across a range of neurological pathologies, we perform a systematic scoping review to identify all clinical studies measuring BBB water exchange using MRI-based techniques in the human adult population.

Methods

Our search protocol was developed according to Preferred Reporting Items for Systematic reviews and Meta-analyses extension for Scoping Reviews (PRISMA-ScR).[258]

We planned to include studies conducted in healthy volunteers, normal ageing and in known pathology.

To be included, studies were required to measure water exchange across the BBB for the purpose of quantifying BBB dysfunction, to be available in English language, involve human adults, perform single-slice or multi-slice imaging acquisition and present whole brain and/or region of interest (ROI) measurements of BBB dysfunction. If applicable, this would also include studies directly comparing water exchange measurement with existing BBB measurements such as PET, DCE-MRI, blood tests or cerebrospinal fluid analysis. Search parameters were limited to studies published after 01/01/2000, in line with the expected clinical availability of MRI technology and our scoping searches.

We excluded clinical studies which focussed on water diffusivity, transepithelial or choroid plexus water exchange. Whilst abstracts were included, given the relative infancy of BBB water exchange measurements, we excluded those whereby the results had later been published as a manuscript and included in this review. Preclinical *in*-vitro and *in-vivo* animal studies were excluded from the main analysis but were reviewed for context and reference searching.

Following several initial scoping searches, we performed a highly sensitive search of MEDLINE, Scopus, EMBASE, CINAHL and Web of Science databases on 05/12/2023,

alongside manual searches of PubMed and study references. The search strategy encompassed 3 main concepts of BBB, MRI and water exchange (see Appendix 3-1 for sample MEDLINE search).

The final search results were exported to EndNote and duplicates removed by MM. Initial screening of title and abstract was completed by MM, escalating any queries to LP and HE. Full texts were subsequently reviewed and presented to the wider study team to determine final inclusion, followed by individual review. Disagreements were settled by consensus and discussion with other co-authors, if necessary. A data-charting table was agreed by MM, LP and HE and the results were discussed and updated as part of an iterative process. We extracted data regarding MRI technique (e.g., ASL, DCE-MRI or FEXI), participants (number, age, morbidity), water exchange measurements, main study outcomes and limitations.

We acknowledge that the clinical translation of water exchange imaging is an emerging field and therefore a scoping review methodology was deemed most appropriate to map the existing data, understand the limitations and interpret this to generate hypotheses for future research themes and strategies. Studies were grouped according to methodology/pathology. Where a systematic review was identified, studies were cross referenced to ensure inclusion.

From our initial searches we identified significant heterogeneity within the available data. Therefore, no meta-analysis was planned.

Results

Database searching revealed 2433 articles, with no further articles identified through cross-referencing and scoping searches. After removal of 226 duplicates, this left 2207 articles for

further screening. Review of title and abstract excluded 2152 articles, leaving 55 full text records for review. Following evaluation and presentation of studies to the wider research team, we included 38 clinical studies (see Figure 3-1).

The 17 studies that were excluded after full text review were due to the following reasons: 9 were not measuring BBB water exchange imaging (e.g. measuring transcellular exchange, water exchange between blood and CSF at the choroid plexus or alternative imaging technique),[217,223,259-265] 2 were hypothesis papers,[175,225] 2 were conference abstracts later published as full manuscripts (which are included in this review),[266,267] 1 was a conference abstract with insufficient information included,[268] 1 was a paediatric feasibility study (though will be included within the discussion),[124] 1 involved animals[269] and 1 was a proof of concept study.[270]

Of the 38 studies included, 28 reported results from ASL imaging - including 15 DW-ASL, 4 Water Extraction with Phase Contrast Arterial Spin Tagging (WEPCAST), 2 Intrinsic Diffusivity Encoding of Arterial Labelled Spin (IDEALS), 2 multi-TE ASL, 1 contrast enhanced ASL and 1 utilising MTFAIR - 8 studies reported DCE-MRI and 4 studies reported FEXI outcomes. This includes 1 study each comparing DCE-MRI with ASL and DCE-MRI with FEXI.

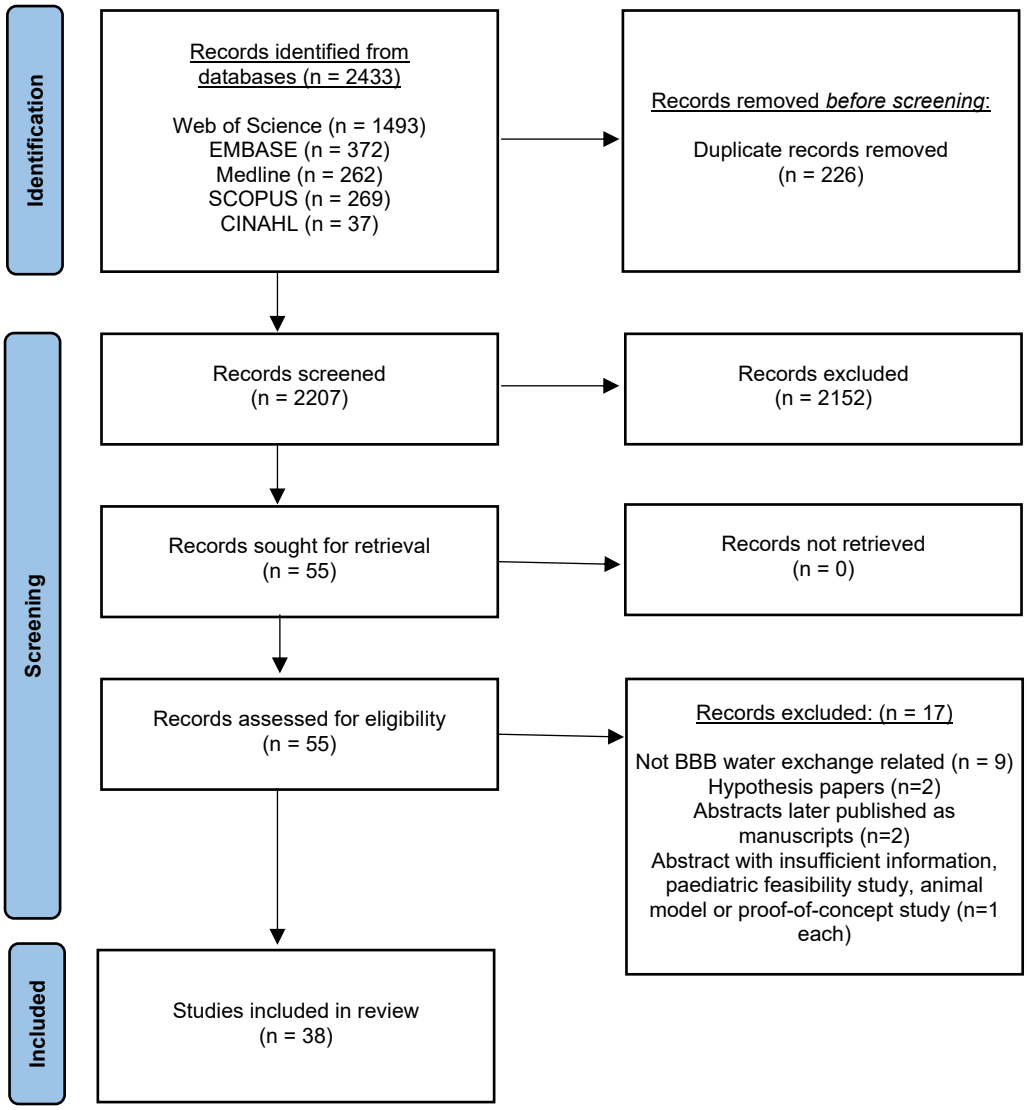


Figure 3-1: PRISMA diagram detailing study identification, screening and inclusion.

Healthy Volunteers / Controls:

We identified 25 studies (total n=364 participants) which either primarily recruited healthy volunteers or included a healthy control group (see Table 3-2).

We review the 15 studies focusing solely on healthy volunteers here; those reported as a healthy control population for comparative purposes are included within their respective section later in this review.

In 2002, Parkes and Tofts demonstrated the first application of a 2-compartment model using continuous (C)ASL data to measure BBB water exchange, hypothesising that water spent longer in the intravascular compartment prior to exchange.[212] Previous studies based upon ASL for the measurement of perfusion were based upon the Kety 1-compartment model, assuming that the brain capillaries were infinitely permeable to water and that water equilibrium was reached instantaneously between the intra- and extravascular compartments.[271] Values produced for white matter (WM) and grey matter (GM) BBB water exchange rate (PS/V_{bw}) were 2.93 and 19.0 ml water $(min)^{-1}$ (ml tissue)$^{-1}$ respectively but there was significant intra-subject variability when the measurement was repeated in a single subject. This is likely to be explained in part by their field strength of 1.5T and the relatively modest difference between blood and tissue T1 relaxation impairing the signal to noise ratio.

St Lawrence *et al.* reported a study of DW-pCASL in 2012, utilising a 2-stage approach to measure BBB water exchange in 7 healthy subjects.[255] They were able to determine both K_w (BBB water exchange rate, equal to PS/V) and arterial transit time (ATT) by combining various diffusion weighted gradient strengths and post labelling delays (PLDs). They employ a PLD time of 1500ms to interpret water exchange, longer than the transit time for water to reach the voxel of interest (capillary space). Post-acquisition processing requires an estimated blood volume (V_b), which was pragmatically determined according to the available literature. Average K_w values for GM (110 ± 18 min^{-1}) and WM (126 ± 18 min^{-1}) agreed favourably with values reported from PET imaging.[181]

Hales *et al.* utilised DWI and the intravoxel incoherent motion model to determine V_{bw} in a study of 10 healthy volunteers, utilising these derived values in a 2-compartment ASL model to estimate PS_w at 1.5T. They demonstrated a mean GM PS_w of 108 ± 2 ml/100g/min.[272]

Authors highlighted the concerns surrounding the assumption of V_{bw} in standard 2-compartment ASL modelling, due to expected regional differences and alterations associated with both neuronal activation and underlying pathology and suggest this method as an alternative. Intra-subject voxel-wise reproducibility measurements were determined to be insufficient for clinical use, but these improved significantly with reduced spatial resolution.

Ford *et al.* performed a modified DW-ASL sequence (with Quantitative Permeability Mapping) in 30 volunteers from a range of age groups, between 25 and 65+ years old. Overall K_w was lower in WM (75.19 ± 13.85 min^{-1}) compared to GM (81.51 ± 15.54 min^{-1}). These values are lower than those previously reported, potentially due to cohort heterogeneity, acquisition parameters or post-acquisition analysis. Authors suggest that direct comparison between the various DW-ASL protocols in the same subjects is required to explain this discrepancy. Age was identified as a statistically significant factor upon K_w in all 5 selected regions (e.g., cerebral cortex, white matter, hippocampi, orbitofrontal and precunei cortices) when adjusting for sex and the number of vascular risk factors present, with overall BBB water exchange lower among older adults. They reported a negative correlation between both white matter hyperintensity (WMH) K_w and WMH volume and between normal appearing (NA)WM K_w and WMH volume (r= -0.51, p=0.02 and r = -0.44, p=0.05 respectively).

Shao *et al*. compared motion-compensated DW-pCASL (MCDW-pCASL) - providing a higher SNR and spatial resolution with 3-compartment modelling - with standard DP-pCASL imaging. A total of 11 participants were included, with 9 undergoing both protocols. Whole brain K_w was 137.8 ± 12.9 and 142.9 ± 9.9 min^{-1} for MCDW-pCASL and DP-pCASL respectively, with good to excellent agreement observed between the two techniques (ICC – 0.82 for K_w). They report PS_w was 61.4% higher in GM (151.6 ml/100g/min) than WM (93.8

ml/100g/min) using the 3-compartment model, in agreement with PS_w measured by PET.[181] Authors also recommend that MCDW-pCASL may be suitable for assessment of the glymphatic system, given the PLD is greater than the ATT (therefore, the measurement of water exchange is taking place at the level of the capillary).

Mahmud *et al*. report a 7T ASL study of 18 healthy volunteers, exploiting the difference in magnetisation transfer (MT) effects in intravascular and extravascular water (due to the presence of macromolecules in brain tissue) to measure BBB permeability.[273] Not previously demonstrated in human studies, they use a flow-sensitive alternating inversion recovery (FAIR) QUIPSS II ASL approach (for details, please see Wong *et al.*[274]) with additional MT saturation pulses to acquire perfusion signals in the presence and absence of macromolecular saturation. They report that PS_w was higher in GM (171 ± 20 ml/100g/min) compared to WM (95 ± 18 ml/100g/min) $p<0.05$ with good reliability in re-test experiments, however authors highlighted that the imaging produced was 2D and further work is required to fully characterise the ability to detect subtle, regional differences in BBB water exchange.

Mahroo *et al.* utilise multi-TE T2-w ASL to measure BBB exchange of water, referred to as T_{exch}.[256] They highlight the potential overestimation of BBB water exchange due to tissue transit effects (for example, whilst labelled water is traversing the arterioles prior to reaching the capillaries) and extend their 2-compartment model to account for 'intra-voxel transit time'. Indeed, their extended model T_{exch} values were 32.6% lower than those calculated via the standard 2-compartment model. Good levels of reproducibility were seen for intra-session, inter-session and inter-visit analyses (Coefficient of Variation, CoV 6.6%, 7.9% and 8.4% respectively).

Mahroo *et al.* further compared 2 multi-TE ASL approaches in 2 groups of healthy volunteers (younger group: 18 ± 1y vs older group: 56 ± 4y). The more complex method (T_{ex}) was a physiologically informed biophysical model estimating the time for water to exchange across the BBB, compared to a simpler method based upon the difference in T2 transverse relaxation to compartmentalise labelled water (K_{lin}).[257] Both methods detected a significant reduction in BBB water exchange in grey matter values of older participants (T_{ex}: younger group 224 ± 51 ms, older group 143 ± 30 ms, K_{lin} younger group 0.150 ± 0.038 s^{-1}, older group 0.054 ± 0.039 s^{-1}), with authors concluding that BBB permeability increased with healthy aging.

Powell *et al.* report a simulation and proof of concept study of CE-ASL, performed upon 6 healthy volunteers (age range 24-46y).[214] Voxel-level estimates of K_b were not feasible in simulations or *in vivo*, though ROI analysis was possible and in keeping with literature values. Their sensitivity analysis identified the optimal post-contrast blood T1 at 3T to be 0.8s with simulations demonstrating that K_b could be estimated in individual cortical regions with a relative error of <1% and co-efficient of variation 30%. They were able to achieve this optimal post-contrast T1 with only a quarter of the clinical dose of GBCA – potentially mitigating concerns regarding renal function and accumulation of contrast agents with longitudinal studies. More precise estimates of K_b were achieved with lower exchange rates – potentially attributed to the model not accounting for the impact of GBCA extravasation decreasing the difference in post-contrast T_1 between blood and tissue. Authors suggest that CE-ASL would therefore be particularly applicable to early, subtle BBB dysfunction states.

Wengler *et al.* report a novel 3T study of IDEALS in 15 healthy volunteers.[275] IDEALS provides whole brain mapping of water permeability without requiring the administration of exogenous contrast agent. The water extraction fraction (E_w) was significantly lower in GM

than WM (E_w GM 78.8 ± 3.3%, WM 83.9 ± 4.6%, p<0.05) whilst PS_w was significantly higher in GM than WM (PSw GM 131.7 ± 29.5 ml/100g/min, WM 76.2 ± 18.4 ml/100g/min, p<0.05). Whilst E_w was reported to be lower in females than males in both GM and WM (p<0.05), PS_w was only significantly different in WM. Authors acknowledge one major limitation of IDEALs is potential instability of the deconvolution process. Furthermore, IDEALs is based upon the assumption that water exchange across the BBB has completed by the time of image acquisition. However, upon varying the post-labelling delay time between 2000ms and 2500ms there they found no significant difference between E_w suggesting that, at least in young healthy volunteers, water exchange has been completed by this time.

In 2018, Lin *et al.* report 4 experiments utilizing WEPCAST, obtaining a global value of BBB water exchange based upon water molecules in the superior sagittal sinus (SSS). An initial WEPCAST protocol in n=6 healthy participants revealed E_w 95.5 ± 1.1% and PS_w 188.9 ± 13.4 ml/100g/min. Utilising a 'look locker' method to shorten the image acquisition time from 19 to 5 minutes returned values of E_w 96.1 ± 1.2% and PS_w 203.3 ± 17.5 ml/100g/min. In a separate cohort of n=6, they performed both WEPCAST protocols and showed good agreement between values (correlation coefficient E_w: 0.87 and PS_w 0.92). A mild hypercapnia challenge demonstrated significantly improved SNR, suggesting this may increase the sensitivity of this technique, whilst not causing any reported discomfort to participants. Whilst presenting the technical feasibility of this technique, in a clinically acceptable timeframe, WEPCAST is limited by lack of regional information which may be important for application in focal pathologies such as neoplastic disease. Furthermore, in healthy volunteers it is expected that approximately 95% of all ASL spins will be extracted into the tissue (limiting the number of detected spins within the SSS and reducing precision). This is likely to be a further limitation

with increased water exchange states, whereby even smaller numbers of MRI visible spins can be expected in the SSS.

Lin *et al.* further evaluated single and multi-delay 3T WEPCAST with comparison to contrast based methodologies alongside test-retest analysis.[203] There was strong correlation between the single and multi-post-labelling delay techniques (R = 0.82, p=0.004), indicating that the shorter scanning protocol remained accurate. Analysis of variance analysis revealed no difference in E_w, cerebral blood flow (CBF) or PS_w between intra- and intersession results (p=0.94, 0.57 and 0.40 for E_w, p=0.45, 0.75 and 0.06 for CBF and p=0.59, 0.78 and 0.47 for PS_w). Interrater reliability was good with an interclass correlation co-efficient 0.90 (95% CI 0.85 – 0.93, p<0.01). There was significant correlation between PS_w derived from WEPCAST and the contrast agent-based technique (R=0.73, p=0.02).

In 2022, Lin *et al* further reported a WEPCAST study to examine the effects of ingesting 200mg of caffeine upon BBB water exchange parameters in 10 healthy volunteers.[276] They found cerebral blood flow reduced in a time dependent manner (p<0.001), E_w significantly increased (p<0.001) but the PS_w remained constant (p=0.94). This suggests that whilst the ingestion of caffeine, an adenosine antagonist causing vasoconstriction and increased neural activity, results in altered perfusion there is no impact upon BBB water exchange. This contrasts with Mahmud *et al* and Wengler *et al* who both reported a reduced PS_w following caffeine administration alongside reduced CBF.[273,275]

Using 3T FEXI, Bai *et al.* reported AXR in 7 healthy volunteers.[224] Two protocols were implemented with varied filter block (b_f) values of 250 s/mm^2 and 900 s/mm^2. Utilising the shorter b_f, expected to effectively filter the fast intravascular pseudo-diffusivity and allow for

modelling of BBB water exchange, authors report AXR and vascular efflux constants (K_{bo}) values in agreement with literature values (K_{bo} GM 4.51 ± 0.70, WM 3.27 ± 0.76 s^{-1}). The longer b_f was thought to instead target transcellular exchange and authors show no significant correlation between exchange rates at different b_f values, supporting these are measuring independent mechanisms.

Powell *et al* perform 3 models of FEXI analysis at 3T in a group of 10 healthy volunteers, with repeat imaging to quantify the accuracy, precision and repeatability of BBB water exchange measurements.[226] Authors recognise the promising results of FEXI thus far, but raise limitations in the interpretation due to 1-compartment analysis and not accounting for the inherent impact that exchange effects have upon T1 and T2 relaxation times. They therefore perform a) an AXR model b) a two-compartment model representing intra- and extra-vascular signal components (2CM) and c) a two-compartment model accounting for finite compartmental T1 and T2 relaxation times ($2CM_r$). Exchange rates in WM and GM were significantly lower in the AXR model compared to 2CM and $2CM_r$ (p<0.001 all comparisons) but there was no significant difference between 2CM and $2CM_r$ (p=0.65/0.82 in WM/GM). Whilst accuracy was highest in the compartmental models, precision was best in the AXR model. Repeatability coefficients for WM/GM were as follows: RC_{AXR} = 0.29 s^{-1}/0.43 s^{-1}, RC_{2CM} = 0.44 s^{-1}/0.51 s^{-1} and RC_{2CMr} = 0.52 s^{-1}/0.61 s^{-1}.

In general, outcomes of healthy volunteer studies were heterogeneous, relating to small participant numbers (range 3 - 36), age, field strength (1.5 – 7.0 T) and methodology. Both acquisition parameters and post-acquisition processing, modelling and interpretation were generally well reported, but varied significantly between studies (as expected with exploratory research). Studies were often limited by either whole brain values or reporting of limited ROIs.

No study demonstrated accurate and reliable voxel-wise analysis of BBB water exchange, with the current imaging resolution appearing most suited to larger ROIs. We note that whilst water exchange values appear similar, error margins remain significant and there is overall little consensus reached between studies – for example, a similar number of studies reported BBB water exchange in GM > WM as those which reported WM > GM (see Table 3-2). We have not synthesised the data here to provide direct comparisons between studies with measures of variability, given the heterogeneous methodologies and the requirement to assume additional parameters (e.g. requiring V_b to calculate K_w from PS_w) – though we note this has been performed previously by Dickie *et al* using literature values where necessary.[173]

Older Participants and Cognitive Impairment:

Six studies recruited older participants (see Table 3-3, total n=203 participants). Pragmatically, we classified these as studies with a mean participant age of over 60 years, reflected by the practice identified by our scoping searches. Naturally, these studies therefore reflect a more diverse population with varying age, vascular risk factors (e.g., smoking status, hypercholesterolemia, diabetes mellitus), polypharmacy and cognitive performance expected.

Gold *et al.* studied the relationship between DW-pCASL derived BBB water exchange and a CSF biomarker of AD (Aβ42 concentration) in 39 cognitively normal older participants (mean age 72.7 years)[277]. They report significant positive correlation between CSF Aβ42 concentration and whole brain K_w values (β=0.51, p=0.002). Correlation was also observed between K_w and CSF Aβ42 in the frontal lobe (p=0.002), parietal lobe (p<0.001), precuneus (p=0.004) and temporal lobe (p=0.015), but not in the medial temporal lobe (p=0.236). This would support the hypothesis that lower K_w is associated with lower CSF Aβ42 – reflecting higher cerebral burden of Aβ42 due to deposition. There was no significant association

between K_w in any of the ROIs and CSF t-tau and p-tau concentrations, nor any significant association between K_w and cognitive performance.

To the contrary, Zachariou *et al.* performed DP-pCASL on 47 healthy older participants (age 70.6 ± 5.54 years) and reported significant correlations between BBB K_w and both executive and episodic memory function.[278] However, the direction of these associations varied between brain regions. For example, voxel-wise linear regression showed K_w was positively associated with cognitive performance in the frontoparietal brain regions, but a negative association was seen in the basal ganglia. The authors suggest this may be due to different physiological underpinnings in the neocortex compared to subcortical structures - perhaps explaining the lack of consistency in the direction of K_w change with cognitive decline in previous studies.

Lin *et al.* utilized 3T WEPCAST in 55 participants (age 68.4 ± 7.3 years), encompassing 33 participants with mild cognitive impairment (MCI) and 22 age-matched controls to evaluate investigate the association between BBB permeability to water, CSF biomarkers, vascular risk factors and cognition.[221] There was no significant difference in age, sex or education levels between groups. Overall, MCI was associated with increased water permeability, with PS_w 142.6 ± 25.6 compared to 123.0 ± 26.0 ml/100g/min (p=0.01). No significant association was found between WMH volume and PS_w. However, BBB PS_w demonstrated a significant inverse correlation with episodic memory (β= -0.0108, p=0.011) and composite cognitive score (β= -0.0051, p=0.041) – i.e., a higher BBB permeability to water was associated with impaired cognition. An inverse trend between increased PS_w and language score and MoCA were also identified, though not reaching statistical significance (p=0.053 and p=0.16 respectively). Interestingly, elevated PS_w was also associated with lower CSF Ab42/Ab40 ratio (β= -0.00027, p=0.0037) suggesting higher levels of CNS amyloid deposition with increased BBB

permeability to water. Higher PS_w was also associated with increased CSF p-tau level (β= 0.45, p=0.012) but not total tau level (p=0.27). No correlation was demonstrated between CSF biomarkers for AD and CSF/serum albumin ratio, but significant correlations were seen between CSF/serum albumin ratio and composite vascular risk factor score (p=0.012) and hypercholesterolaemia (p=0.011). Authors suggest that whilst increased small molecule (i.e., water) permeability is correlated with declining cognitive performance and markers of CNS amyloid deposition, permeability to larger molecules (i.e., albumin) is instead associated with vascular risk factors – implicating a difference in sensitivity to the underlying pathophysiology.

In an abstract, Anderson *et al.* reported a study of 5 older participants (age 74 ± 6 years) using 3T DCE-MRI to compare BBB water exchange with choroid plexus water exchange.[279] They report a strong positive correlation between transepithelial water exchange at the choroid plexus with PS_w in the hippocampus (R=0.84, p=0.07), suggesting that exchange processes at the choroid plexus may influence the wider brain microvasculature permeability.

Anderson *et al.* also report a 7T DCE-MRI based study observing BBB water exchange in WM ageing with 38 older participants in 2020.[280] Whilst V_b showed minimal changes over the 40 year age span of participants, they report a decline in K_{po} by 0.06 s^{-1}/year (p<0.0005) which remained significant when adjusted for WM volume. This suggests compromised neurovascular unit metabolism with ageing. The authors acknowledge that the relationship between K_{po} and age are theoretically unlikely to be linear and may indeed reflect participant selection.

Zhang *et al* compared 3T FEXI (referred to in the study as vascular exchange imaging or VEXI) derived BBB water exchange measurements between 3 groups; 11 with AD, 14 with MCI and

27 age-matched cognitively normal control participants.[281] There was no significant difference in age, gender and education level between groups. They report higher AXR values in the MCI group when compared to controls, specifically within the hippocampus. AXR values were further increased in the AD group compared to MCI, but the abnormalities were more widespread extending to the thalamus and medial orbital frontal cortex. Linear regression modelling revealed significant, negative correlations between AXR in all 3 brain regions and MoCA score in all subjects (r= -0.44, p=0.001 in hippocampus, r= -0.50, p=0.0002 in thalamus and r= -0.53, p<0.0001 in the medial orbitofrontal cortex) but only the correlation in the hippocampus remained significant when the AD group was removed from the analysis (r= -0.32, p=0.043) – suggesting that AXR increase is associated with cognitive decline but perhaps only within the thalamus and medial orbitofrontal cortex at a later stage of disease.

Overall, studies within the older population reported heterogeneous results but with a greater focus on cognitive performance and ROI BBB water exchange. It is unlikely that there will be a truly linear relationship between age and BBB water exchange, given the number of confounding variables, but larger studies are required to further establish this trend. Additional research is also required to understand the relationship between water exchange and CSF AD biomarkers, especially with the recent advances in treatment options available for AD patients.

Cerebral Small Vessel Disease (cSVD):

We identified 7 studies which evaluated BBB water exchange in participants with cSVD (see Table 3-4, total n=209 participants).

Shao *et al.* initially presented an abstract in 2018 utilising DW-pCASL in a cohort of 20 older subjects (age 68.8 ± 7.6 years) at risk of cSVD.[282] Participants underwent 2x 3T MRI scans

approximately 2 weeks apart, alongside blood tests, physical examination and neuropsychological evaluation. Average K_w was significantly correlated with ATT (p<0.01), Picture Sequence Memory Test (measures episodic memory) (p=0.02) and 4m Walk Gait Speed (p=0.04). K_w was 23.8% higher in subjects with diabetes (118.3 ± 7.6 min^{-1}) compared to those without (95.6 ± 12.4 min^{-1}), with multiple regression models of K_w with age, gender, hypertension, cholesterol and diabetes status supporting the finding of increased K_w in diabetic patients (p<0.01). They report an ICC of K_w between repeated scans of 0.77.

Shao *et al.* later published the wider results as a manuscript in 2019, having performed DP-pCASL in 19 older participants at risk of cSVD (age 68.8 ± 7.6 years).[215] They utilised a novel algorithm to estimate K_w, proposing this as an improved mathematical framework for de-noising or under sampled reconstruction. Authors report that K_w was increased by 28.2% in participants with diabetes (p<0.001) and 19.5% in those with hypercholesterolaemia (p=0.04). K_w was higher in participants with higher vascular risk factors (p=0.02), with decreased neurocognitive performance (e.g., Dimensional Change Card Sort Test – p=0.02, Picture Sequence Memory Test p=0.03 and p<0.001 for test *a* and *b* respectively). Whilst they did show a positive correlation between K_w and WMH volume, this did not reach statistical significance (p=0.20). Scans were repeated approximately 2 weeks apart to assess reproducibility, with ICC for whole brain K_w = 0.75 reducing to ICC~0.5-0.75 in most ROIs (lower in smaller regions such as the hippocampus and parahippocampal gyrus).

Fujima *et al.* further performed DW-ASL for the investigation of brain WMH progression.[216] They recruited 41 consecutive participants (mean age 67.5 years) who were referred for an MRI brain, upon which there was evidence of WMHs and they had previous imaging available for comparison within the prior 2 years They formed two groups, ‘progressive’ or ‘non-

progressive', based upon the comparative size of WMH or the appearance of new lesions within ROIs between their respective scans. They also recruited 5 healthy controls (mean age 62.8 years) without WMHs for comparison. They defined the *normal* K_w level as the mean value for all ROIs in these control subjects. The K_w values in the progressive WMH group (109.6 ± 28.2 min^{-1}) were not significantly different from the non-progressive group (105.1 ± 8.1 min^{-1}) p=0.42. However, in the progressive group, a lower CBF and higher FLAIR severity was noted. Acknowledging that both higher and lower K_w have been implicated in BBB dysfunction, they also calculated variance in K_w ($K_{w\text{-}var}$) by squaring the difference between the ROI K_w and the normal K_w value. This revealed that $K_{w\text{-}var}$ was significantly higher in the progressive ROIs (735.4 ± 736.3 $[min^{-1}]^2$) compared to the non-progressive ROIs (88.7 ± 130.5 $[min^{-1}]^2$) p<0.001. Results were limited due to the heterogeneous participant profiles and only a small number of control participants on which the normal K_w values were calculated. Furthermore, modelling was based upon fixed ATT values, whilst some alteration in large artery integrity may be expected between groups due to, for example, vascular stenosis.

Li *et al*. report a case-control study to investigate the role of BBB dysfunction in 2 of the most prevalent forms of genetic cSVD, namely cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and heterozygous HTRA1 mutation-related cSVD.[283] Participants (24 CADASIL, 9 HTRA1) underwent DP-pCASL, conventional MRI sequences to measure cSVD burden and a clinical cognitive assessment. Compared to 24 healthy controls, K_w was reduced in CADASIL and HTRA participants in both whole brain and multiple brain ROIs. For comparison, mean whole brain K_w was 133.19 ± 18.16, 117.31 ± 23.70 and 95.13 ± 26.17 min^{-1} in healthy controls, CADASIL and HTRA1 groups respectively. In the CADASIL group, decreased K_w in whole brain (β= -0.634, p=0.001), normal appearing WM (β= -0.599, p=0.002) and temporal lobe (β= -0.654, p=0.001)

was significantly associated with higher cSVD score after adjusting for age and sex. Reduced whole brain K_w was significantly associated with poorer neuropsychological performance after adjusting for age, sex and education in both the CADASIL and HTRA1 groups (β=0.458, p=0.001; β=0.884, p=0.008). Authors suggest that DP-pCASL may be suitable for monitoring the disease course of genetic cSVD and further research is required into the exact underlying mechanism for the reduction in K_w.

Ling *et al.* report a further study of DP-pCASL in 41 participants with CADASIL compared to 36 age and sex matched controls.[284] K_w was reduced in the NAWM (t= -4.742, p<0.001), cortical GM (t= -5.137, p<0.001) and deep GM (t= -3.552, p=0.001) in the CADASIL group when compared with controls. When adjusted for age, sex and ATT, K_w in NAWM was negatively associated with the volume of WMHs, whilst decreased K_w in NAWM was independently associated with an increased risk of abnormal mRS scale score (Odds ratio, OR=1.058, 95% confidence internal, CI: 1.013-1.106, p=0.011). Authors conclude that BBB water exchange is reduced in CADASIL, with decreased BBB water exchange associated with conventional MRI burden of cSVD and functional dependence. They also suggest that DP-pCASL could be utilised as a non-invasive evaluation for disease severity.

In a conference abstract, Yunqing *et al.* compared DP-ASL with DCE-MRI in 39 participants with CADASIL and 40 healthy controls.[285] They reported K_w was reduced in multiple brain regions in those with CADASIL, whilst there was no significant difference in K_{trans}. They conclude that the mechanism underlying BBB dysfunction measured by K_w and K_{trans} is different, with no significant correlation between values in any brain region.

Shao *et al.* performed DP-pCASL and DCE-MRI at 3T to directly compare BBB water exchange rate with the BBB permeability to and exchange rate of GBCAs (K_{trans}, K_{Gad}) in a cohort of elderly participants at risk of cSVD (n=16, age: 67.9 ± 3.0 years).[286] They assessed test-retest reproducibility with repeated scans approximately 6 weeks apart. Authors report that significant correlation between K_w and K_{trans} was only found in WM (β=6.7 x 10^4, p=0.036), caudate (β=8.6 x10^4, p=0.029) and middle cerebral artery (MCA) perforator territory (β=6.9 x 10^4, p=0.009), but not in whole brain, GM, medial temporal lobe, amygdala, hippocampus or parrahippocampal gyrus. Significant correlation was identified between K_w and K_{Gad} in MCA perforator territory (β=1.5 x10^3, p=0.049), medial temporal lobe (β=3.5 x 10^3, p=0.032) and hippocampus (β=3.4 x 10^3, p=0.038), but not the remaining brain regions. Good reproducibility of K_w measurements (ICC=0.75) was achieved. The modest correlation between GBCA extravasation and BBB water exchange measurements further support the hypothesis of differing mechanisms of BBB dysfunction.

In conclusion, cSVD studies show a trend towards reduced BBB water exchange acknowledged in genetic forms of cSVD such as CADASIL and HTRA1, with little correlation in water exchange values and BBB permeability to GBCAs. This is in agreement to a previous DCE-MRI study, which showed no difference in K_{trans} in genetic cSVD, but did find a difference in sporadic cases.[29] However, we note that Uchida *et al.* report contrasting results, whereby a higher regional K_{trans} was measured in patients with CADASIL, when compared to controls.[287] Further studies are also required to understand the significance of concepts such as $K_{w\text{-}var}$ and the influence of co-morbidities such as diabetes mellitus or hypercholesterolaemia upon results.

Multiple Sclerosis (MS):

Four studies were identified including patients with MS (see Table 3-5, total n=56 participants), including 2 studies of Relapsing Remitting (RR)MS and 2 studies of Progressive (P)MS. Three studies utilized ss-DCE-MRI and 1 study used IDEALS.

Relapsing Remitting Multiple Sclerosis (RRMS):

Rooney *et al.* utilized 7T ss-DCE-MRI to compare participants with RRMS with non-enhancing WMHs (n=6) to healthy controls (n=6).[156] Overall, water exchange in RRMS subjects was reduced in non-enhancing WMHs compared to NAWM (K_{po} 1.8 ± 0.45 vs 2.2 ± 0.20 s^{-1} respectively). However, water exchange was also reduced when comparing NAWM in the MS subjects to WM in controls (K_{po} 2.2 ± 0.20 vs 3.2 ± 0.56 s^{-1}) and similarly in NAGM of MS subjects to GM in controls (K_{po} 2.0 ± 0.13 vs 2.9 ± 0.59 s^{-1}). This supports the theory that MS is a whole brain, diffuse process. Authors acknowledge that contrast extravasation in MS lesions is usually increased, despite demonstrating reduced BBB water exchange – hypothesising this is due to altered neurovascular metabolic activity. However, many participants were early in the disease course and longitudinal data is required to confirm this. In 1 late-stage participant, with extensive WMH burden, whilst K_{po} continued to be significantly reduced in WMHs, K_{po} was actually increased in NAGM – with this postulated as a potential biomarker for conversion to secondary progressive MS (e.g., indicating increased 'detour' circuitry or reflective of an increased rate of apoptosis). Again, larger study would be required to investigate this finding further.

Wengler *et al.* studied RRMS (n=11) using 3T IDEALS, with comparison to healthy controls (n=14).[288] Initially, 15 RRMS participants were recruited, but 4 were excluded for having MS lesions below the resolution of IDEALS parameter maps (4x4x4 mm^3). For analysis, all WMHs

were grouped (limiting the appreciation of the heterogeneous nature of MS lesions), but nil demonstrated contrast enhancement to suggest active disease. Significantly lower PS_w and CBF was reported in WMHs compared with NAWM (ΔPS_w -11.5 ml/100g/min, $p<0.05$ and ΔCBF -8.1 ml/100g/min, $p<0.05$). Lower PS_w and E_w were reported in NAWM in the RRMS cohort vs WM in controls (ΔPS_w: -11.9 ml/100g/min, $p<0.05$, ΔEw: -4.3%, $p<0.1$), and lower E_w in NAGM vs GM (ΔE_w: -12.1%, $p<0.01$). This further substantiates the diffuse metabolic dysfunction in MS, which authors suggest may be due to mitochondrial function or the reduced metabolic requirements of the surrounding brain parenchyma. However, no correlation was found between changes in BBB water exchange measurements and the Estimated Disease Severity Scale (EDSS)[289] – though a larger sample size would be required for the study to be adequately powered for this parameter.

Progressive Multiple Sclerosis (PMS):

In a published abstract, Spain *et al.* performed ss-DCE-MRI in 16 participants with PMS in comparison to 14 healthy controls. They report a significant reduction in K_{po} in NAWM and NAGM when compared to WM and GM in controls ($p<0.05$), with V_b only significantly increased in NAWM ($p<0.0005$).[290] Values for BBB water exchange were not included in this abstract. Authors suggest that this reduction in K_{po}, which they suggest is indicative of reduced metabolic function, may be an important component of brain atrophy in PMS.

The same group later reported a larger study of 7T ss-DCE-MRI in participants with PMS (n=23) compared to healthy controls (n=19).[291] They demonstrate a reduction in K_{po} in WMHs (1.93 ± 0.71 s^{-1}) compared to NAWM (2.83 ± 0.70 s^{-1}) $p<0.0001$, but no significant difference between PS_w in WMHs and NAWM (0.063 ± 0.038 vs 0.052 ± 0.009 ml/g/s, $p=0.2$). In NAWM, there was no significant difference in K_{po} between groups. However, in NAGM, K_{po}

was significant reduced in PMS compared to healthy controls (p=0.01). The authors report decreased transcapillary water exchange in lesions may reflect the impact of chronic inflammation on energy metabolism and ion homeostasis. Participants with Primary Progressive MS and Secondary Progressive MS were grouped as PMS, which could impact on the outcomes – however there was no significant effect when adjusted for age, sex, disease duration or PMS subtype on any parametric estimation

Together, there appears to be an emerging trend towards reduced water exchange measurements within WMHs in both RRMS and PMS, but further preclinical studies are required to fully understand the role of endothelial cell and surrounding brain parenchymal metabolic function on transcapillary water exchange. Furthermore, longitudinal clinical studies are required to understand the alterations of water exchange with active lesion formation (e.g., new contrast enhancing WMLs compared with non-enhancing WMLs) and disease course – including the impact of disease modifying treatment. Longitudinal studies should be sufficiently powered to consider clinical parameters such as EDSS, cognitive measures and transition to SPMS.

Brain Tumours:

Four studies have reported water exchange measurements in participants with brain tumours, summarized in Table 3-6 (total n=29 participants).

Wang *et al.* initially used 3T DW-pCASL to evaluate a single patient (age 21 years) with a grade II oligodendroglioma, alongside 13 healthy controls (mean age 24.6 years).[292] Increased PS/V_c was demonstrated in both the growing area (463.4 min^{-1}) and solid tumour (347.8 min^{-1}) when compared with the surrounding grey matter (223.6 min^{-1}). This finding correlated with

post-contrast enhancement, but caution should be taken when interpreting these results in a single subject as changes in V_c and ATT associated with the underlying neoplastic process may lead to similar findings.

As part of their wider study including RRMS participants and healthy controls, Rooney *et al.* also evaluated 5 participants with glioblastoma multiforme (GBM) using 3T ss-DCE-MRI.[156] K_{trans} measurements of GBCAs were increased in GBM, whilst K_{po} was significantly reduced (K_{po} ≤0.18 s^{-1} in tumour tissue compared with 3.2 and 2.9 s^{-1} in WM and GM respectively for healthy controls). As expected in a focal pathology, relatively normal values were obtained from the remaining brain parenchyma. Reduced K_{po}, in the context of elevated K_{trans}, is likely to represent either reduced NVU metabolic integrity or indeed reduced metabolism of the tumour. Longitudinal data would be required to understand the changes in water exchange with tumour growth, as well as in differing regions of the tumour, for example in the presence of a necrotic core region.

Bai *et al.* reported 3T ss-DCE-MRI measures in biopsy proven GBM participants (n=10).[208] They reported increased intra-lesional contrast extravasation when compared to NAWM (K_{pe} 3.0 x 10^{-2} s^{-1} vs 6.1 x 10^{-4} s^{-1}, p<0.001) in the context of reduced water exchange (K_{bo} 1.0 s^{-1} vs 2.8 s^{-1}). This further supports the theory that water and GBCAs are probing different BBB pathways. However, overall, the amount of water being exchanged remains significantly higher than the amount of GBCA leakage – reflecting the relatively high flux of the active and passive component of water exchange. Authors indicate that whilst an increase in the paracellular leakage of water is expected – this is likely to be diminished by the significant reduction of active water transport due to metabolic dysfunction.

Wang *et al.* report ss-DCE-MRI and FEXI measurements in 13 participants (age 58.4 ± 9.4 years) with high grade glioma (4 grade III, 9 grade IV), finding a reduction in BBB water exchange values within the tumour compared to NAWM and NAGM utilising both methods.[254] Indeed, values measured by either method were highly comparable. Authors suggest that this reduced water exchange may be due to altered neurovascular coupling, downregulation of aquaporin-4 in gliomatous tissue or reduced transendothelial transportation (e.g., Na^+-K^+-ATPase pump activity).

Brain tumours remain a highly heterogeneous population, with the role of factors such as growth, specific histology, hormone/growth factor receptor status, blood supply, treatment (e.g., radiotherapy, chemotherapy and corticosteroids) and presence of vasogenic oedema requiring additional consideration. Furthermore, studies have thus far only considered primary brain neoplastic disease; no study was identified evaluating secondary or metastatic disease. Longitudinal studies with histological correlates are required in the clinical domain to further evaluate the role of water exchange in brain tumour and whether this can assist with significant clinical questions such as marking the transformation from low-grade to high-grade glioma. Whole brain BBB water exchange values are less likely to be as helpful as ROI or voxel-wise analysis given the focal nature of the underlying pathology.

<u>Other Pathologies:</u>

We identified 3 further studies relating to BBB water exchange in participants with heart failure, obstructive sleep apnoea (OSA) and schizophrenia.

Shinnick *et al.* reported 3T DW-pCASL measurements of BBB water exchange in the brains of participants with heart failure (n=3, age 52 ± 19 years) compared with 6 healthy controls

(age 53 ± 3 years).[293] Global K_w values were lower in participants with heart failure (87.8 ± 13.5 min^{-1}) when compared with controls (105.3 ± 21.5 min^{-1}), but this did not reach statistical significance p=0.2.

Palomares *et al.* evaluated subjects with OSA (n=9, age 46.7 ± 10.5 years) in comparison to healthy controls (n=9, age 38.8 ± 6.8 years), also using 3T DW-pCASL.[294] There was no significant difference in body mass index between groups (p=0.38). They report lower global K_w values for OSA participants in WM (p=0.006) and GM (p=0.002) when compared with controls, however ATT and CBF remained similar. Authors indicate compromised BBB function, potentially contributing to the neurodegeneration seen in OSA, but intact large artery integrity.

Goldwaser *et al*. utilise DP-ASL to study compare BBB water exchange between patients with schizophrenia spectrum disorder (n=32) with healthy controls (n=27).[295] They also examined peripheral vascular endothelial health via brachial artery flow-mediated dilation to understand whether centrally measured K_w is related to endothelial function. Whole brain average K_w and peripheral endothelial function was significantly reduced in the schizophrenia group (p=0.007, p=0.0001 respectively). Reduced K_w in the right superior corona radiata and right parietal angular gyrus were associated with negative symptoms of schizophrenia. This supports the hypothesis that altered neurovascular mechanisms are underlying schizophrenia, especially the negative symptoms. Authors also report that age was no associated with whole brain average K_w, but older age was generally associated with lower K_w and there was no significance difference between males and females.

Overall, whilst studies are limited by small numbers and varied pathology, there are signals to suggest that BBB dysfunction may be seen in conditions not traditionally considered *neurological* such as heart failure or obstructive sleep apnoea – though we note the increased prevalence of neurological symptoms in this cohort.[296-299] Further study is required to confirm and better characterise this finding, with consideration to whether altered haemodynamic parameters such as ATT, CBF or V_b could be contributory to these findings.

Discussion:

MRI studies of BBB water exchange have been reported in healthy volunteers, ageing, AD, MCI, cSVD, MS, brain tumours, heart failure, OSA and schizophrenia. Though not included in this review – due to being a paediatric cohort – we are aware of the WEPCAST-based feasibility study reported by Lin *et al*. detailing the increased BBB permeability to water in 21 participants with sickle cell disease compared to 5 siblings with sickle cell trait.[124]

Measurement of BBB dysfunction has a potential role in the early diagnosis, prognosis and monitoring of a variety of neurological disorders, alongside showing promise as a surrogate end point for trials of novel therapeutics.[96] With an ageing population and both significant morbidity and mortality associated with neurological conditions, alongside the increasing roll-out of disease modifying therapies and a shift towards personalised medicine, there has never been a more pressing time for such technology.[300-302] Given the considerable advantages of MRI-based BBB water exchange measurements, such as the existing clinical availability of hardware, lack of patient exposure to ionising radiation and without requiring exogenous contrast agent administration in most protocols, it is prudent to prioritise further validation of these methods.

Promisingly, animal studies have shown changes in BBB water exchange rate prior to detection with DCE-MRI in AD, as well as changes in water exchange associated with ageing and infection.[202,269,303-305] Mechanisms such as tight junction disruption, altered endothelial and surrounding parenchymal metabolic activity alongside differing expression of perivascular aquaporin-4 have been implicated.[208,254,269,303] Indeed, a human post-mortem study revealed increased expression of aquaporin-4 in AD and cerebral amyloid angiopathy.[306] But BBB water exchange remains incompletely understood and is complicated by factors such as CO_2 levels and caffeine administration, which have been shown to influence measured values and require additional consideration.[275,276,307] Further studies are urgently required to also understand the role of the glymphatic system, blood-CSF barrier at the choroid plexus (the site of CSF production) and any compensatory interactions between these.[134]

Translation into the clinical setting also presents additional variables – patient demographics or clinical parameters such as age, sex, co-morbidity and cognition may impact upon measurements, as might physical parameters such as arterial stenosis changing ATT/CBF values.[204,275,281,308] Scanning duration will need to be carefully balanced with consideration to the clinical availability of hardware, patient tolerability and workable SNRs to answer the relevant clinical question. We note several techniques present a whole brain value for BBB water exchange which often results in a reduced scan times, but we are mindful that this may be less applicable to focal pathologies such as neoplasm where values may increase and decrease in different regions.[278] Indeed, resolution at a ROI or, in some cases, voxel-wise level may be required and this has not yet been reliably achieved in many brain regions.

Facilitating this progress will require large-scale, longitudinal studies to establish normal and pathological values within the population, with direct comparisons between modalities (e.g.,

ASL, FEXI, DCE-MRI and potentially probing specific BBB transporters with nuclear imaging) and standardised protocols. Longitudinal imaging has, thus far, usually been for calculation of repeatability with short intervals, rather than measurement of the natural history of an underlying pathology. Combining modalities would also provide an assessment of bias when assuming values such as blood volume – which is likely to be regionally different, both physiologically and pathologically, for example due to angiogenesis in the context of brain tumour and dynamic changes brough about by neuronal activation.[272] Standardising the acquisition parameters, image processing and the reporting values is required – ideally with data shared collaboratively between study teams.

This review does have limitations – many of which are associated with the early, exploratory nature of the research included. We have focussed on only water exchange measurements of BBB dysfunction – though we do note the ongoing work with relation to other novel markers such as BBB glucose imaging.[159] We have deliberately not dwelled on the technical imaging acquisition parameters or post-processing, highlighting instead the various clinical applications of these techniques – but we have signposted several reviews to this effect. Given the heterogeneity of included methods, we have not performed data synthesis or meta-analysis and instead aim to map the available evidence for the purposes of hypothesis generation.

In conclusion, BBB water exchange studies utilising MRI have been reported across a range of neurological pathologies with a potential role in diagnostics, disease monitoring and therapeutics. However, whilst alterations in BBB water exchange have been reported in several studies, the underlying pathophysiology requires additional consideration and present studies are predominantly small, with heterogenous methodologies and lack longitudinal data. Further research into this potentially valuable method is recommended.

Author	Year	Technique	Field strength	GBCA	No. healthy controls (mean age - years)	Whole brain - mean	WM - mean	GM - mean
Parkes *et al.*[212]	2002	cASL	1.5T	-	n=3 (28y)	-	PS/V_{bw} 2.925 ml water$(min)^{-1}(ml\ tissue)^{-1}$	PS/V_{bw} 19 ml water$(min)^{-1}(ml\ tissue)^{-1}$
Wang *et al.*[292]	2007	DW-pCASL	3T	-	n=13 (26.4y)	-	PS/V_c 166 ± 55 min^{-1} (from single subject)	PS/V_c 189 ± 56 min^{-1} (from single subject)
Shinnick *et al.*[293]	2014	DW-pCASL	3T	-	n=6 (53 ± 3y)	K_w 105.3 ± 21.5 min^{-1}	-	-
St Lawrence *et al.*[255]	2012	DW-pCASL	3T	-	n=7 (28 ± 5y)	-	K_w 126 ± 18 min^{-1}	K_w 110 ± 18 min^{-1}
Hales *et al.*[272]	2013	DWI, IVIM & ASL	1.5T	-	n=10 (24y)	-	-	PS 108 ± 2 ml/100g/min
Palomares *et al.*[294]	2015	DW-pCASL	3T	-	n=9 (38.8 ± 6.8y)	-	K_w 261.1 ± 51.0 min^{-1}	K_w 220.8 ± 40.6 min^{-1}
Ford *et al.*[309]	2022	DW-ASL	3T	-	n=30 25–44y – n=9 45–64y – n= 8 ≥65y – n=13	-	75.19 ± 13.85 min^{-1}	cortical GM: 81.51 ± 15.54 min^{-1}
Li *et al.*[283]	2023	DP-pCASL	3T	-	n=24 (42.88 ± 12.34y)	K_w 133.19 ± 18.16 min^{-1}	K_w 120.30 ± 16.94 min^{-1}	-
Ling *et al.*[284]	2023	DP-pCASL	3T	-	n=36 (45.03 ± 11.71y)	-	Kw 124.82 ± 19.68 min^{-1}	Kw CGM: 116.61 ± 14.71 min^{-1} Kw DGM 108.79 ± 17.57 min^{-1}

Shao *et al.*[310]	2023	MCDW-pCASL / DP-pCASL	3T	-	n=11 (26 ± 3y)	MCDW-pCASL: K_w 137.8 ± 12.9 min^{-1} DP-pCASL: K_w 142.9 ± 9.9 min^{-1}	<u>3 compartment model:</u> K_w 106.7 min^{-1} PS_w 93.8ml/100g/min E_w 92.2%	<u>3 compartment model:</u> K_w 126.3 min^{-1} PSw 151.6 ml/100g/min E_w 94.7%
Mahmud *et al*[273]	2023	MT-FAIR (ASL)	7T	-	n=18 (27 ± 11y)	-	E 0.962 ± 0.015 PS 95 ± 18 ml/100g/min)	E 0.921 ± 0.025 PS 171 ± 20 ml/100g/min)
Mahroo *et al.*[256]	2021	multi-TE ASL	3T	-	n=10 (range 28 - 40y)	-	-	T_{exch} 227.9 ± 37.9 ms
Mahroo *et al.*[257]	2023	multi-TE pCASL	3T	-	<u>Younger group:</u> n=13 (18 ± 1y)	-	-	T_{ex} 224 ± 51 msec
					<u>Older group:</u> n=13 (56 ± 4y)	-	-	T_{ex} 143 ± 30 msec
Powell *et al.*[214]	2023	CE-ASL	3T	Dotarem 2 x 0.025 mmol/kg	n=6 (age range 24-46y)	-	-	K_b 2.32 ± 2.49 s^{-1}
Wengler *et al.*[275]	2019	IDEALS	3T	-	n=15 (28 ± 9y)	-	E_w 83.9 ± 4.6%, PS_w 76.2 ± 18.4 ml/100g/min	E_w 78.8 ± 3.3%, PS_w 131.7 ± 29.5 ml/100g/min
Lin *et al.*[307]	2018	WEPCAST	3T	-	n=23 in total across experiments (26 ± 6y)	<u>WEPCAST:</u> E_w=95.5 ±1.1% PS_w 188.9 ± 13.4 mL/100g/min <u>LL-WEPCAST</u> Ew 96.1 ± 1.2% PSw 203 ± 17.5 ml/100g/min	-	-

Lin *et al.*[221]	2021	WEPCAST	3T	Gadolinium diethylenetriamine pentaacetic acid.	n=10 (experiment 1) (24.8 ± 4.0y)	<u>Single delay:</u> E_w 92.9 ± 2.8% <u>Multi-delay:</u> E_w 94.0 ± 2.0%		
Lin *et al.*[276]	2022	WEPCAST	3T	-	n=10 (29.1 ± 9.3y)	E_w 91.0 ± 3.5 % PS_w 133.6 ± 16.9	-	-
Bai *et al.*[224]	2020	FEXI	3T	-	n=7 (24 ± 2y)	-	K_{bo} 3.27 ± 0.76 s^{-1}	K_{bo} 4.51 ± 0.70 s^{-1}
Powell *et al.*	2023	FEXI	3T	-	n=10 (range 23 – 52y)	-	AXR: 2.10 ± 0.39 s^{-1} 2CM: 3.11 ± 0.43 s^{-1} $2CM_r$: 2.95 ± 0.27 s^{-1}	AXR: 1.53 ± 0.47 s^{-1} 2CM: 2.23 ± 0.46 s^{-1} $2CM_r$: 2.27 ± 0.49 s^{-}
Rooney *et al.*[156]	2015	ss-DCE-MRI	3T	Gadoteridol 28µmol/kg	n=6 (30 ± 10y)	-	K_{po} 3.2 ± 0.56 s^{-1}	K_{po} 2.9 ± 0.59
Tagge *et al.*[291]	2021	ss-DCE-MRI	7T	Gadoteridol 14µmol/kg	n=19 (52.7 ± 11.7y)		K_{po} 3.28 ± 0.85 s^{-1}	K_{po} 1.98 ± 0.42s^{-1}

Table 3-2: 25 studies included healthy volunteers, either as the main focus of the study or as a control population. Abbreviations: GBCA: Gadolinium based contrast agents, WM: White matter, GM: Grey matter. *Spain *et al.* 2017[290] [abstract], Wengler *et al.* 2020[288] and Goldwaser *et al.* 2023[295] did not report raw BBB water exchange values and are not presented here.

Author	Year	Technique	Field Strength	GBCA	No. older participants (mean age)	Whole brain – mean	WM – mean	GM – mean
Gold *et al.*[277]	2021	DW-pCASL	3T	-	n=39 (67 – 86y)	K_w 98.27 ± 19.77 min^{-1}	-	-
Lin *et al.*[203]	2021	WEPCAST	3T	-	n=22 (68.5 ± 6.2y)	PS_w 123.0 ± 26.0	-	-
Anderson *et al.*[280]	2020	DCE-MRI	7T	Gadoteridol <0.05mmol/kg	n=38 (60.7 ± 12.8y)	-	K_{po} 2.1 ± 1.1s^{-1}	-

Table 3-3: 6 studies included older participants, with mean age >60 years old. Abbreviations: GBCA: Gadolinium based contrast agent, WM: White matter, GM: Grey matter.

*Anderson *et al.* 2013[279] [abstract], Zhang *et al*. 2023[281] and Zachariou *et al.* 2023[278] did not report raw BBB water exchange values and are not included here.

Author	Year	Technique	Field	GBCA	Pathology	No. cases (age)	Whole brain - mean	WM - mean	GM - mean
Shao *et al.*[282]	2018	DW-pCASL	3T	-	Older participants, at risk of cSVD	<u>With diabetes:</u> n=7 (68.7 ± 4.2y)	K_w 118.3 ± 7.6 min^{-1}	-	-
						<u>Without diabetes:</u> n=13 (68.8 ± 9.1y)	K_w 95.6 ± 12.4 min^{-1}	-	-
Shao *et al.*[215]	2019	DW-pCASL	3T	-	Older participants, at risk of cSVD	n=19 (68.8 ± 7.6y)	K_w 105.0 ± 20.6 min^{-1}	K_w 94.1 ± 19.6 min^{-1}	K_w 109.6 ± 19.6 min^{-1}
Shao *et al.*[286]	2020	DCE-MRI / pCASL	3T	Gadoterate meglumine 0.1 mmol/kg	Older participants, at risk of cSVD	n=16 (67.9 ± 3.0y)	K_{trans} 6.6 ± 0.7 x10^{-4} min^{-1} K_{Gad} 1.9 ± 0.3 x10^{-2} min^{-1} K_w 122.3 ± 16.5 min^{-1}	K_{trans} 6.1 ± 1.1 x10^{-4} min^{-1} K_{Gad} 3.3 ± 0.7 x10^{-2} min^{-1} K_w 121.9 ± 17.2 min^{-1}	K_{trans} 6.8 ± 0.7 x10^{-4} min^{-1} K_{Gad} 1.6 ± 0.2 x10^{-2} min^{-1} K_w 122.6 ± 15.6 min^{-1}
Fujima *et al.*[216]	2020	DW-pCASL	3T	-	WMHs / chronic ischaemia/ leukoaraiosis	n=41 (67.5y)	<u>K_w in ROIs:</u> Progressive group: 109.6 ± 28.2 min^{-1}	-	-
							Non-progressive: 105.1 ± 8.1 min^{-1}	-	-
Li *et al.*[283]	2023	DP-pCASL	3T	-	CADASIL	n=24 (44.17 ± 14.14y)	-	K_w 102.96 ± 20.91 min^{-1}	-
					HTRA1	n=9 (42.78 ± 15.14)	-	K_w 90.81 ± 25.98 min^{-1}	-
Ling *et al.*[284]	2023	DP-pCASL	3T	-	CADASIL	n=41 (44.61 ± 10.61y)	-	K_w 102.25 ± 20.86 min^{-1}	K_w CGM: 94.62 ± 22.46 min^{-1} K_w DGM: 95.04 ± 16.37 min^{-1}

Table 3-4: 7 studies have included participants with cerebral small vessel disease. Abbreviations: GBCA: Gadolinium based contrast agent, WM: White matter, GM: Grey matter. *Yunqing *et al.* 2023[285] [abstract] did not include raw BBB water exchange values and is not reported here.

Author	Year	Technique	Field strength	GBCA	Pathology	No. cases (age)	No. controls (age)	WMH	NAWM	NAGM	WM	GM
Rooney *et al.*[156]	2015	ss-DCE-MRI	7T	Gadoteridol 28µmol/kg	RRMS	n=6 (46 ± 7y)	n=6 (30 ± 10y)	K_{po} 1.8 ± 0.45 s^{-1}	K_{po} 2.2 ± 0.20 s^{-1}	K_{po} 2.0 ± 0.13 s^{-1}	K_{po} 3.2 ± 0.56 s^{-1}	K_{po} 2.9 ± 0.59 s^{-1}
Tagge *et al.*[291]	2021	ss-DCE-MRI	7T	Gadoteridol 14µmol/kg	PPMS / SPMS	n=23 (57 ± 6.7y)	n=19 (52.7 ± 11.7)	K_{po} 1.93 ± 0.71 s^{-1}	K_{po} 2.83 ± 0.70 s^{-1}	K_{po} 1.64 ± 0.41 s^{-1}	K_{po} 3.28 ± 0.85 s^{-1}	K_{po} 1.98 ± 0.42 s^{-1}
Wengler *et al.*[288]	2020	IDEALS (ASL)	3T	Gadolinium based	RRMS	n=11 (40.9 ± 10.9y)	n=14 (39.7 ± 11.3y)	PS_w 53.9 mL/100g/min	PS_w 82.1 mL/100g/min	-	-	-

Table 3-5: 4 studies reported water exchange outcomes in patients with multiple sclerosis (MS). Abbreviations: GBCA: Gadolinium based contrast agent, WMH: White matter hyperintensities, WM: White matter, GM: Grey matter, NAWM: normal appearing WM, NAGM: Normal appearing GM. **Spain et al. 2017[290] [abstract] not listed due to insufficient data included, however K_{po} reported to be significantly lower in NAWN and NAGM in PMS compared to healthy controls ($p<0.05$) when using ss-DCE-MRI. Wengler et al. values are based upon a single participant's reported values, otherwise values were reported as $\Delta PS_w/E_w$.*

Author	Year	Technique	Field strength	GBCA	Pathology	No. cases	Tumor	WM	GM
Wang *et al.*[292]	2007	DW-pCASL	3T	-	Grade II oligodendroglioma	n=1	PS/V_c growing area 463.4, solid tumour 347.8 min^{-1}	-	PS/V_c 223.6 min^{-1}
Rooney *et al.*[156]	2015	ss-DCE-MRI	3T	Gadoteridol 28µmol/kg	Glioblastoma multiforme	n=5	K_{po} <0.18 s^{-1}	Frontal WM K_{po} 0.44 s^{-1}	-
Bai *et al.*[208]	2020	ss-DCE-MRI	3T	Gd-DTPA 0.1mmol/kg	Glioblastoma multiforme	n=10	K_{bo} 1.0s^{-1} K_{pe} 3.0x$10^{-2}s^{-1}$	K_{bo} 2.8s^{-1} K_{pe} 6.1x$10^{-4}s^{-1}$	-
Wang *et al.*[254]	2023	ss-DCE-MRI (Kbo) and FEXI (AXR)	3T	-	High grade glioma (4 grade III, 9 grade IV)	13	K_{bo} 1.03 ± 0.75s^{-1} AXR 1.94 ± 1.04s^{-1}	K_{bo} 3.50 ± 0.59s^{-1} AXR 3.35 ± 0.77s^{-1}	K_{bo} 2.10 ± 0.56s^{-1} AXR 2.07 ± 0.52s^{-1}

Table 3-6: 4 studies have investigated the impact of brain tumour on water exchange rate. Abbreviations: GBCA: Gadolinium based contrast agent, WM: White matter, GM: Grey matter.

Chapter 3 - Appendix List:

Chapter 4: Method – Water EXchange in the vasculature of the BRAIN (WEX-BRAIN): An exploratory study of blood-brain barrier water exchange imaging in patients with cerebral small vessel disease.

Chapter 4:

Contribution statement:
The WEX-BRAIN study is a £1 million multi-centre Engineering and Physical Sciences Research Council (EPSRC) funded project led by Prof Geoff Parker (Centre for Medical Image Computing, University College London) in collaboration with Prof Laura Parkes (University of Manchester) and Prof Hedley Emsley (Lancaster University, Lancashire Teaching Hospitals NHS Foundation Trust).

WEX-BRAIN is a two-phase project, beginning with preclinical development and optimisation of water exchange imaging techniques, followed by trialling these novel methods in a small group of participants with cerebral small vessel disease.

My involvement is limited to this second, clinical phase of the WEX-BRAIN study which is included here.

I drafted, revised and finalised the study protocol based on my Chapter 1 literature search and discussions with the wider WEX-BRAIN team. I applied for and obtained ethical approval (NHS & university panels), represented the study at the REC meeting, applied for Lancashire Teaching Hospitals sponsorship, for NIHR portfolio adoption and study recruitment/scanning site set up at Lancashire Teaching Hospitals, Northern Care Alliance (Salford Royal Hospital) and at the Manchester Clinical Research Facility. I created all of the study documents.

I was responsible for participant screening/identification, consent, data collection and provided medical supervision for the MRI scanning visits. I processed the white matter hyperintensity volumes, brain volumes and performed statistical analysis of the data.

Due to computational requirements, the BBB-FEXI processing was completed by the WEX-BRAIN research team at University College London but I was responsible for data analysis as presented in Chapter 5.

Distribution:
This protocol has not been submitted for publication on consensus decision by the wider research team.

Clinical WEX-BRAIN Protocol:

Researchers:	**Dr Mark Maskery (PhD student)** Senior Clinical Research Fellow (Neurosciences) – Royal Preston Hospital, Lancashire Teaching Hospitals NHS Foundation Trust Out of Programme Research (OOP-R) – Specialty Training – Neurology – North West Deanery. Department of Neurology, Royal Preston Hospital, Sharoe Green Lane, Preston, Lancashire, PR2 9HT Mark.Maskery@lthtr.nhs.uk
Investigators:	**Prof. Hedley Emsley (PhD lead supervisor / Chief Investigator / Principal Investigator)** Consultant Neurologist and Professor of Clinical Neuroscience – Royal Preston Hospital, Lancashire Teaching Hospitals NHS Foundation Trust and Lancaster Medical School, Lancaster University. Department of Neurology, Royal Preston Hospital, Sharoe Green Lane, Preston, Lancashire, PR2 9HT Hedley.Emsley@lthtr.nhs.uk **Prof Laura Parkes (PhD co-supervisor / Lead for imaging methodology and Principal Investigator for Phase I of WEX-BRAIN)** Professor of Neuroimaging - Division of Neuroscience and Experimental Psychology, University of Manchester. **Dr Dwaipayan Sen (Principal Investigator – Patient Identification Centre)** Consultant in Stroke Medicine and Aging and Complex Medicine, Comprehensive Stroke Centre, Manchester Centre for Clinical Neurosciences, Salford Royal Hospital.
Recruitment sites:	**Primary:** Lancashire Teaching Hospitals NHS Foundation Trust (PI – Prof Emsley) **Participant Identification Centre:** Salford Royal NHS Foundation Trust (PI – Dr Sen)
Funding:	Engineering and Physical Sciences Research Council (EPSRC) grant funding (EP/S031367/1 and EP/S031510/1)
Sponsor:	Lancashire Teaching Hospitals NHS Foundation Trust

Study Aims, Objectives and Hypothesis:

There is an increasing focus on the role of blood-brain barrier (BBB) dysfunction in the pathophysiology of a range of neurological conditions. Measuring water exchange across the BBB using Magnetic Resonance Imaging (MRI) as a non-invasive, quantitative marker of this process has been proposed.[173]

Originally developed to measure water exchange across cell membranes by exploiting the differences in diffusivity between tissue compartments, FEXI has been adapted and optimised to measure water exchange across the BBB (BBB-FEXI).[25,224]

The primary aim of this clinical phase of the Water EXchange in the vasculature of the BRAIN (WEX-BRAIN) study is to perform an exploratory analysis of BBB-FEXI in a small group of healthy volunteers and participants with cerebral small vessel disease (cSVD).[311,312] By working with our local NIHR Lancashire Clinical Research Facility (CRF), this will also serve secondary aims of priming the local research environment to perform future imaging studies and developing a network of neurovascular, imaging and health data science researchers to foster longer term studies for patient benefit.

We intend to recruit participants with a range of cSVD burden including those with a recent lacunar infarct. This is based upon local expertise, discussions with experts in this field and reflecting the heterogeneous phenotype of the cSVD cohort. Participants will be identified through routine clinical practice, including our recently characterised 2-week wait (2WW) suspected central nervous system (CNS) cancer pathway (see Chapter 2).

Using only clinically available imaging hardware for acquisition, we aim to compare the BBB-FEXI measurements with conventional radiological markers of cSVD such as white matter hyperintensity (WMH) volume and examine for associations with participant characteristics and cognition.[3,96]

We hypothesise that BBB-FEXI will be adequately sensitive to differentiate participants with cSVD from age-matched controls. We hope to explore correlations between BBB-FEXI and age, vascular risk factors (including composite vascular risk scores) and advancing WMH burden but we recognise that the study will not be sufficiently powered to fully investigate these findings.

Primary Research Question:

Is there a difference between BBB water exchange, measured using BBB-FEXI, between participants with cSVD and controls?

Secondary Research Questions:

- Are BBB-FEXI values increased or decreased in participants with cSVD, compared to controls?
- Is there an association between BBB-FEXI values and the burden of cSVD measured by conventional imaging (e.g., WMH volume)?
- In participants with cSVD, is there a difference between BBB-FEXI values within the WMHs and normal appearing white matter (NAWM)? How does the water exchange within NAWM compare with white matter values in controls?
- Is there a difference between BBB-FEXI values between participants with recent stroke, compared to those with cSVD and controls?

- Are BBB-FEXI values associated with patient demographics/characteristics? e.g., age, gender, vascular risk factors, QRISK3 profile or measures of physical dependency?
- Are BBB-FEXI values associated with cognitive function (focussing on executive function / processing speed)? How does this compare with WMH volume?

Materials and Methods:

Participants will be recruited prospectively from Lancashire Teaching Hospitals (LTH) NHS Foundation Trust (in conjunction with the on-site Lancashire NIHR CRF). For a summary of participant recruitment see Figure 4-2

Subjects:

We intend to recruit a total of 80 participants, encompassing 40 participants with a range of cSVD burden and 40 controls who are ideally age-matched (see Figure 4-1).

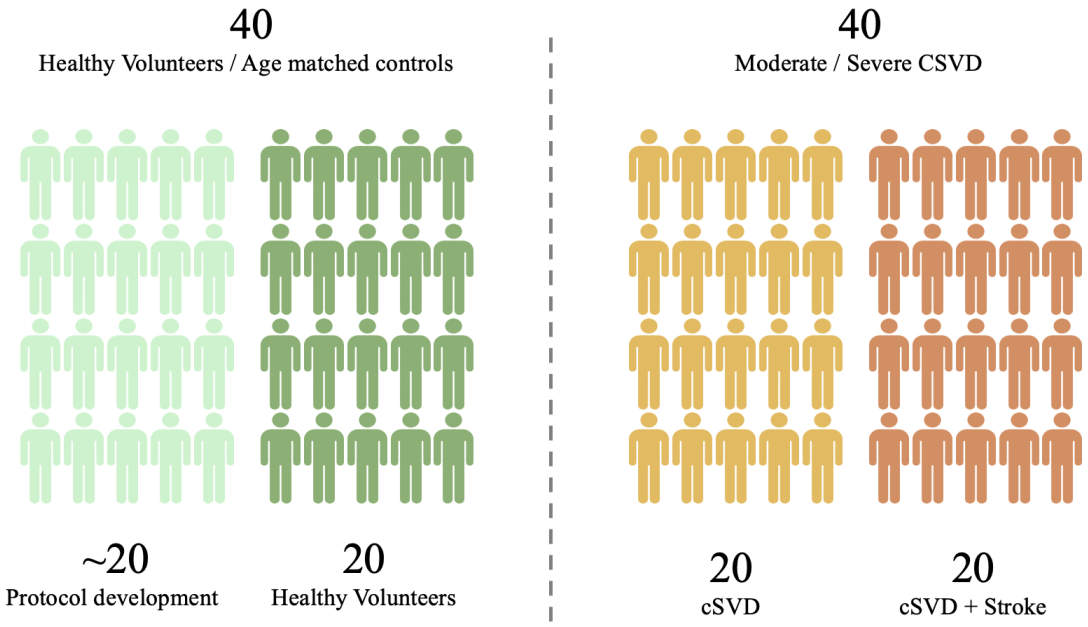


Figure 4-1: Projected WEX-BRAIN participant recruitment

- Cerebral small vessel disease (cSVD) (n=40)
    - Moderate/severe cSVD ***without*** recent stroke (n=20) – recruited from various routine/urgent inpatient and outpatient settings whereby radiological evidence of moderate/severe cSVD has been identified on imaging in the absence of recent clinical stroke or significant structural abnormality (e.g., space occupying lesion). This will include TIA clinic referrals, the regional 2WW suspected CNS cancer multidisciplinary team (MDT) meeting and routine neurological practice.
    - Moderate/severe cSVD ***with*** recent stroke (n=20) – recruited via the local inpatient stroke service and transient ischaemic attack (TIA) clinic at LTH Participants will have experienced a recent (3-12 months) ischaemic stroke with anatomically relevant DWI/ADC changes on a routinely performed MRI brain *and* have background radiological features consistent with moderate/severe cSVD.
- Age/gender matched controls and healthy volunteers (n=40):
    - Healthy volunteers will be recruited through advertisement to university staff and students as well as participants from previous studies who have provided consent to be contacted again. We will also approach potential participants identified through routine clinical practice, whereby imaging has been performed as part of normal management (e.g., for the exclusion of CNS malignancy) in whom there is no significant neurological diagnosis or radiological evidence of cSVD. We envisage that up to 20 of these healthy controls will be scanned prior to the main study to implement and optimise the novel imaging measurements.

Inclusion Criteria:

- Age >18 years
- Fluent in English (to ensure safe supervision of imaging and to support cognitive testing)
- *Only for those receiving intravenous gadolinium-based contrast agents (GBCA):* estimated glomerular filtration rate (eGFR) > 60ml/min/1.73m^2 [313]
- *Presence of moderate/severe cSVD:* for the purposes of defining moderate/severe cSVD, we will adopt a standardised approach using the visual Fazekas rating scale (considering the location and confluence of WMHs).[46]
- *Presence of recent stroke:* we defined a recent ischaemic stroke as an eloquent lesion on DWI/ADC with a corresponding clinical presentation. Both cortical and subcortical strokes will be included as the study nature is inherently exploratory.

Exclusion Criteria:

- Prior history of severe head trauma, meningoencephalitis, epilepsy, brain tumour, schizophrenia, bipolar affective disorder, dementia or neurodegenerative disorder
- History of recreational drug abuse
- Contraindication to MRI or previous reaction to gadolinium[314]

Participant screening:

Potential participants will be identified through several streams as outlined above and screened based upon the inclusion/exclusion criteria. Participants will be then approached either by telephone call or face-to-face discussion and provided with a Participant Information Sheet (PIS). After providing sufficient time for consideration, participants will be contacted again to discuss the study and, if relevant, visit 1 will be scheduled.

Visit 1 - Baseline assessment:

We envisage this baseline assessment will take place as a home visit and be completed within a total time of 1 hour (see Table 4-1 for a summary of assessments and Figure 4-2 for a summary of visits). Where necessary, a clinic room is available at the CRF. The PIS will be re-visited and all questions related to the study will be answered in full. Participants who then provide informed written consent will be enrolled into the study and baseline information will be collected:

- Confirmation of past medical history, allergies and current medications (where available and with permission from the participant, this information can be obtained from hospital/general practitioner (GP) electronic records).
- Measurement of blood pressure, height and weight alongside calculation of Body Mass Index (BMI).
- Assessment of vascular risk factors (e.g., smoking status, family history) and calculation of a QRISK3 score.[315]
- Completion of the latest University of Manchester MRI safety questionnaire
- Where necessary, a blood sample will be collected for measurement of renal function (only required for those undergoing a GBCA-enhanced MRI scan)
- Patient Health Questionnaires (PHQ-2/PHQ-9) for depression screening[316]
    - A score of 3 or greater on the PHQ-2 will prompt the administration of the full PHQ-9 questionnaire to evaluate for evidence of major depression. Should a patient score ≥10 on the PHQ-9, indicative of major depression, participants will be advised to seek review with their GP who will also be informed of these findings by letter. Participants will also be guided to the Lancashire Minds Matter (now 'Lancashire and South Cumbria Talking Therapies') self-referral

service which includes information regarding where to seek urgent, local help if required.[317]

- Cognitive assessments tailored for cSVD (see 'Cognitive testing' below).

Home visits will be conducted in line with the Lancaster University Division of Health Research Lone Researcher policy. This includes access to technology such as SkyGuard if required. Appropriate risk assessments will be completed in line with local policy and any training needs will be addressed to ensure safe working.

A letter will be sent to cSVD participant's general practitioner informing them of enrolment to the WEX-BRAIN study including a copy of the PIS and contact details for the PhD researcher and Principal Investigator.

	Visit 1	Visit 2	Visit 3
Medical questionnaire	**X**		**X**
U&E measurement	**X**		
MRI safety questionnaire	**X**	**X**	
BMI	**X**		**X**
QRISK3	**X**		**X**
PHQ-2/PHQ-9	**X†**		**X†**
Edinburgh Handedness Inventory		**X**	
MRI scan (BBB-FEXI)		**X**	
MoCA	**X**		**X**
BMET	**X**		**X**
UoM online cognitive battery	**X**		**X**

Table 4-1: Assessment schedule. *U&E measurement will only be performed if a GBCA-enhanced MRI scan is planned. †PHQ-9 will only be completed if the PHQ-2 score is ≥ 3.

Visit 2 – Scanning Visit

Participants from the healthy control and cSVD group without a recent stroke will then be invited for the next available MRI scan appointment. Participants with cSVD and a recent stroke will be scheduled for imaging at least 3-12 months following initial stroke presentation (based upon the experience of research team members and the expectation that this will allow for stabilisation of post-stroke haemodynamic and radiological factors). Clinically, this will also ensure that recruitment does not impair the best medical management that patients will undergo as part of routine stroke care (e.g., any requirement for post-stroke rehabilitation). This broad interval will offer the opportunity to consider the optimal timing from initial stroke presentation to guide further studies.

Participants will make a single visit to either the Manchester NIHR/Wellcome Trust Clinical Research Facility based in Manchester or Salford Royal Hospital lasting approximately 90 minutes, where they will undergo an MRI scan at a magnetic field strength of 3.0 Tesla. The

scan will take approximately 1 hour to complete. Participants will receive reimbursement for their time and travel costs, or a taxi will be provided for their return journey.

Upon arrival, the MRI safety questionnaire will be repeated, their height and weight will be confirmed (where contrast is to be administered) and they will be required to remove personal possessions (e.g., watches, credit cards) and any other objects which could affect the operation of the scanner / the safety of the participant. Items will be stored safely in a locker provided. An Edinburgh Handedness Inventory Questionnaire will be performed to indicate their dominant cerebral hemisphere.[318]

One of the potential MRI sequences will involve a GBCA to be injected into the antecubital vein in the arm. When this is to be completed, the radiographer/medical doctor will cannulate the participant's arm and leave the cannula in the vein ready for the contrast agent to be injected during the scan. A medically qualified doctor will be present to oversee this.

A qualified radiographer will take the participant to the MRI scanner and will carry out the scan. The participant will be positioned comfortably on the scanner table with their head in the imaging coil and moved into the imaging tube. The participant will be provided with an alert button to press if they have any problems or wish to communicate with the radiographers and will be informed that they can abort the scan at any time.

Once completed, the participant will be assisted from the scanner and accompanied away from the area.

Visit 3 - Follow up:

We will arrange a further home visit approximately 12-18 months following the scan visit to collect longitudinal data including an interim medical history (e.g., any new diagnosis of dementia, stroke, hypertension), updated vascular risk factor profile and to repeat the cognitive assessments. This opportunity will also be used to provide patients with a newsletter regarding the study progress (which we envisage will also be made available via the Lancashire Neurosciences website[319] or any successor website).

If additional funding is secured, and pending an amendment to the ethics approval, we would also invite selected participants to undergo a repeat MRI scan. These participants will be identified based upon their initial imaging findings, cognitive performance and the outcome of any interim analyses. Where participants agree to a further scan and GBCA is required, a kidney function blood test will be repeated to ensure that eGFR remains within the inclusion criteria. They will then be invited to attend for a further MRI scan at the approved imaging locations.

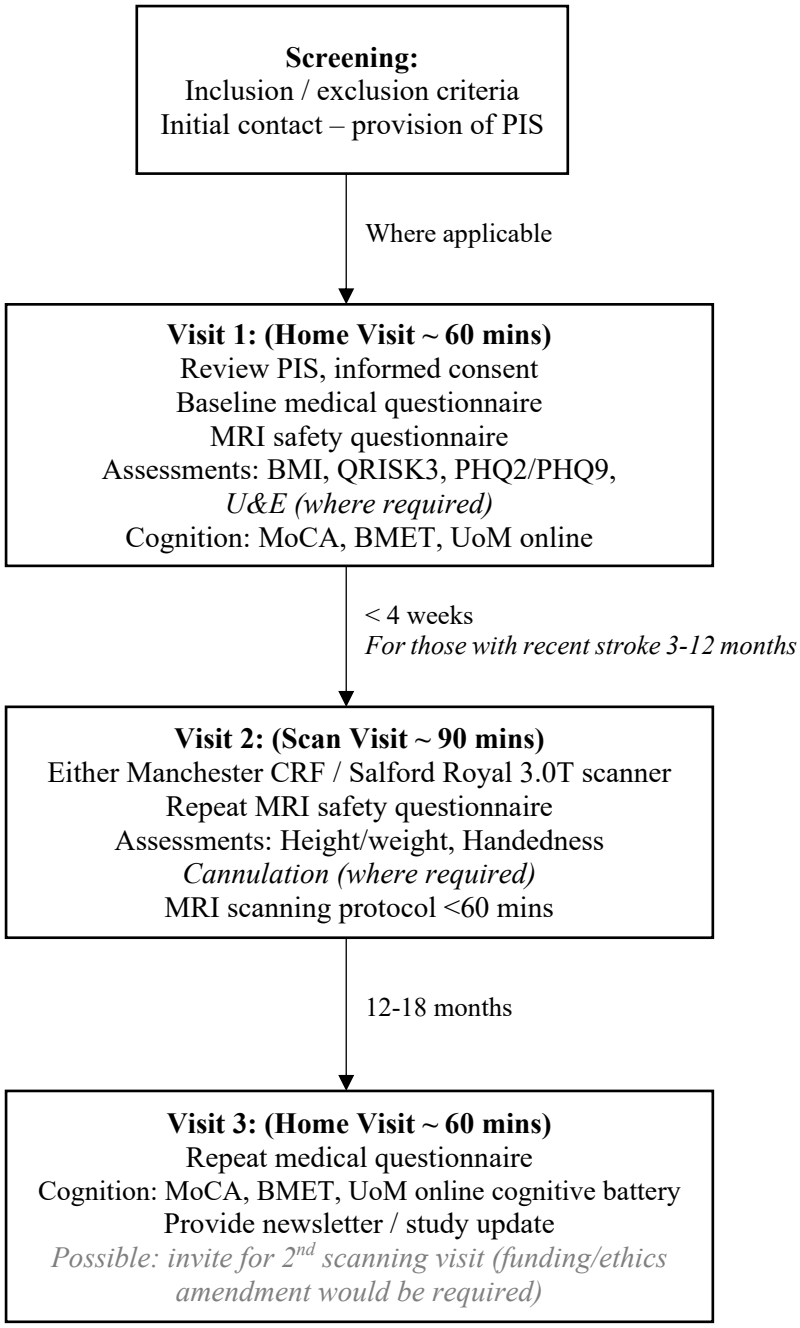


Figure 4-2: WEX-BRAIN participant visit schedule

Imaging Protocol:

The intended MRI protocol will be established prior to study opening and optimised during the initial scanning visits.

Participants will be scanned for a maximum of 1 hour, with the following sequences envisaged:

- High resolution T1-, T2-weighted, Fluid-Attenuated Inversion Recovery (FLAIR) and susceptibility weighted imaging (SWI) sequences for anatomical localisation and exclusion of significant incidental findings, segmentation of grey/white matter, region of interest (ROI) volume analysis and identification/analysis of conventional radiological markers of cSVD.
- T1/T2 mapping for voxel-wise analysis of tissue properties.
- Dynamic contrast enhanced (DCE)-MRI imaging for comparative assessment of BBB dysfunction.
    - **Note:** This sequence will require the supervised injection of GBCA and will only be completed where renal function has been checked and is in accordance with the study inclusion criteria (eGFR > 60 ml/min/1.73m).
- Novel BBB-FEXI sequence (optimised during preclinical study phase of WEX-BRAIN)

<u>Cognitive testing:</u>

The association between vascular cognitive impairment in cSVD and BBB dysfunction is well established, predominantly impacting upon executive functioning and processing speed with relatively intact episodic memory by comparison to typical Alzheimer's disease.[151,320-325] Participants will therefore undergo cognitive testing as part of the baseline and follow up assessments (Visits 1 & 3).

Depression can impact upon cognitive performance; participants will therefore be screened for depression using the validated Patient Health Questionnaire (PHQ)-2. If positive, they will also complete the PHQ-9. The result will be recorded and may be utilised during the analysis of

cognitive function; however, this result will not change participant involvement with the study protocol.

Based upon our current funding we intend to complete the following assessments having considered the time constraints upon visit schedules and reviewing the protocols available for other cSVD studies:[59,326]

- Montreal Cognitive Assessment (MoCA)[327,328]
- Brief Memory and Executive Test (BMET)[329]
- University of Manchester (UoM) online cognitive battery for cerebral small vessel disease – developed by Katie Moran, PhD student at UoM to measure speed and accuracy across 4 cognitive tasks. This can be completed on any computer with a standard UK keyboard.
    - Basic reaction time task – participants will be presented with a series of letters and required to respond by pressing the letter 'x' when they see the letter 'h'.
    - Semantic conceptual processing speed task – participants will be presented with a sentence and asked to assess whether or not the sentence makes sense, indicating 'x' for yes or 'n' for no.
    - Psychomotor processing speed task – participants will be presented with a series of letters, either: *m, l, p, d, w* or *v*, and required to respond by pressing the corresponding key on the keyboard before returning to the 'shift' keys to standardise their beginning position between trials.
    - Visual perception processing speed task – participants will be presented with images of two objects simultaneously and asked to assess whether the images were perceptually the same by pressing the 'x' key for yes and 'n' for no.

If additional funding is available, we will consider alternatively utilising the CANTAB cognitive battery for stroke and cerebrovascular disease (https://cambridgecognition.com/stroke-and-cerebrovascular/).

Risks:

MRI scanning is safe, well tolerated and does not expose patients to ionising radiation.[314] Patients with a contraindication to MRI (e.g. those with metallic implants or a permanent pacemaker in situ) will not be eligible for inclusion. Participants will undergo the University of Manchester MRI safety questionnaire at Visit 1 and this will be repeated prior to entering the scanner at Visit 2.

We know that a small number of individuals will not be able to tolerate an MRI scan (e.g. due to claustrophobia or difficulty lying flat for sustained periods).[330] However, given our methods of recruitment we envisage the majority of participants will have undergone a prior MRI scan and be familiar with the process. Scan time will also be kept to a minimum to optimise participant comfort and experienced research radiographers will complete the MRI scan. Participants will have been provided with an information sheet to explain what will happen on the date of scanning and the opportunity to ask any questions. They will be provided with an alert button during the scan and, if they are unable to tolerate the MRI scan, they will be removed from the scanner tube as soon as possible.

For the cSVD group, the participant's GP will be advised of their recruitment via letter. Healthy participant's GP will only be contacted in the event of incidental radiological findings.

We acknowledge the potential for incidental findings on MR scanning. Many are likely to be of limited significance as we envisage most participants will have recently undergone imaging during their routine clinical care. However, all research MRI scans will be reviewed by a consultant neuroradiologist (likely to be Dr Sachin Mathur or Dr John Cain, Lancashire Teaching Hospitals NHS Foundation Trust) to identify any incidental structural abnormalities. Significant incidental findings will be communicated to the patient's general practitioner by Dr Mark Maskery (Neurology Specialist Trainee) and Prof Hedley Emsley (Consultant Neurologist).

MRI GBCAs are routinely administered to patients during clinical care. The main side effect is slight nausea, but this is infrequent.[331] GBCA administration is considered a safe procedure in most patients, the main risks occurring if the patient has impaired renal function. Participants with known renal impairment at baseline or allergy to GBCA will therefore be excluded from the study or will be scanned without contrast administration.

Where utilised, contrast will be administered via an intravenous cannula; this is a safe procedure and well tolerated. It is likely that participants will have had a cannula inserted previously. There is a small risk of bleeding/bruising and a very infrequent risk of thrombophlebitis.

In the small number of participants invited to undergo repeat scanning, a further blood test will be completed to ensure that kidney function remains satisfactory prior to this assessment.

Analysis Design:

Sample size:

As part of the original funding application, the following power calculation was included:

- *A power calculation has been derived from previous results[303]. In this study, they reported an average difference in PS_w (Permeability surface area of water, a promising marker for BBB permeability) between AD rats and wild type rats of approx. 3 ml/min/ml, with a standard deviation of approx. 2.5ml/min/ml for each group. CSVD patients are expected to show greater BBB dysfunction than seen in AD; we therefore assume an effect size of 5ml/min/ml. If we assume human studies at 3T have approximately half the signal to noise ratio of rat studies at 7T, we may expect a standard deviation of approximately 5 ml/min/ml for each group. This implies that 16 subjects will be required in each group to observe a difference of $p<0.05$ with 80% power.*

This study is exploratory in nature and thus it is difficult to precisely determine the required sample size to ensure sufficient power to test all of our hypotheses. However, we have based our intended sample size of 80 participants pragmatically on the following considerations:

- We aim to recruit a total of 40 healthy volunteers, we envisage a proportion of which will be scanned to optimise the study imaging protocol. This will also allow for age and gender matching, where possible.
- A total of 40 cSVD participants (20 with and 20 without recent stroke) will allow for comparison between groups and provide some resilience to drop out / contingency with translating preclinical model findings.

- Furthermore, this sample size has been considered in line with the available funding and time required for image acquisition and analysis of results during the 3-year PhD period.

Imaging and data analysis:

Incidental findings: Anatomical imaging (T1/T2w, FLAIR, SWI) will be reviewed visually with a consultant neuroradiologist to examine for evidence of significant intracranial findings. Where identified, these will be communicated to the participant's GP.

Brain volume segmentation: T1w imaging will be analysed using Freesurfer to acquire whole brain, grey/white matter and ROI volumes. The finalised protocol will be included in Appendix 4-5.

WMH volume: FLAIR imaging will be analysed using the Lesion Segmentation Toolbox in Matlab to acquire a semi-automated measurement of WMH number and volume. Generated maps will be visually scrutinised to ensure appropriate fitting. The finalised protocol will be included in Appendix 4-6.

DCE-MRI: Where performed, the DCE-MRI sequences will be analysed by the University of Manchester neuroimaging team according to previously published methods.[101]

BBB-FEXI: Due to the experimental nature of this novel technology, the pre-processing and analysis of BBB-FEXI will be performed as per ongoing in-house development, subject to change during the initial healthy volunteer scanning period. We expect this will be analysed by the University College London neuroimaging team.

Statistical analysis: This will be performed using R using appropriate techniques guided by support from the Lancaster University Centre for Health Informatics, Computing, and Statistics (CHICAS).[243] Demographics, clinical characteristics, imaging and longitudinal cognitive data will be compared between the healthy volunteer and cSVD groups using appropriate statistical techniques.

Confidentiality and Data Usage:

All information collected for the WEX-BRAIN study will be kept confidential. All data will be transferred and stored securely in line with all local protocols. Study participants will be assigned a study ID.

Participants will be enrolled after providing informed consent that they have no objection to personal data being used for the purposes of research in this study (in line with the General Data Protection Regulation GDPR and Data Protection Act 2018).

All data stored on computers will be password protected and labelled with the participant's coded, study ID. Personal names or details will never be stored alongside the study data. A document linking personal details to the study ID will be kept on paper in a locked filing cabinet at LTH.

Study data will be accessed by the research team at Lancaster University, University of Manchester and the NIHR Lancashire Clinical Research facility. Anonymised data will be shared with trusted research collaborators using secure means. Participants will not be identifiable in any of the data that we share or analyse.

Anonymous data acquired for this study may be used for public engagement activities and re-analysed in the future, which participants will be asked to consent to.

Public engagement:

The Lancashire NIHR CRF has an agenda for public and patient involvement in all studies. Specific public engagement opportunities have been guided by the wider WEX-BRAIN team as part of the EPSRC funding application.

WEX-BRAIN outcomes will be communicated via a newsletter at Visit 3, via media (e.g., Lancashire Neurosciences website), publication and presentations.

Impact:

We anticipate short-term benefits to the academic community, scanner manufacturers and the pharmaceutical industry, with subsequent benefits for healthcare and patients. We hope that clinicians will be the longer-term end-users of this research, using BBB water exchange measurements to characterise disease early and select/monitor treatment on an individual patient basis.

Epilogue:

The protocol outlined above is based upon the original document submitted for Research Ethics Committee (REC) and Health Research Authority (HRA) approval, receiving a favourable outcome in July 2021.

With sponsorship from Lancashire Teaching Hospitals, REC/HRA approval and NIHR portfolio adoption we were able to obtain the *green light* from our imaging sites (Northern Care Alliance and NIHR Manchester Clinical Research Facility) in November 2021 and recruited our first participant shortly afterwards on 3rd December 2021.

Unfortunately, on 8th December 2021, additional COVID-19 restrictions were introduced due to the rapid spread of the Omicron variant and, alongside NHS winter pressures, significantly limited our ability to recruit and scan further participants.[332] It was in February 2022 that the national COVID-19 restrictions were lifted as part of the 'Living with COVID-19' strategy, but a significant proportion of outpatient consultations continued to operate on a remote basis.

By April 2022, WEX-BRAIN recruitment remained static (see Figure 4-3). It was clear that COVID-19 had changed the recruitment landscape.[333-337] The COVID-19 lockdown mindset was still prevalent, with potential participants reluctant to accept home visits, to travel unnecessarily or to visit healthcare establishments due to concerns regarding increased risk of infection. A large study of patients with rheumatological conditions echoed these changes in behaviour.[338] Many participants were taking part in COVID-19 related research studies already. Participants were also anecdotally more reluctant to receive contrast agent, citing recent press coverage of COVID-19 vaccination side effects as an indication for potential unexpected consequences.

We also recognised an unexpected difficulty in recruiting participants from the cSVD cohort. On several occasions patients were seemingly unaware of the presence of cSVD on their recent imaging and had understood their investigation had returned 'normal', 'unremarkable' or 'reassuring'. Understandably, this raised questions and required careful consideration and

discussion to avoid undue concern, but often resulted in potential participants less willing to enrol in a study for a pathology of which they previously had no knowledge of. This was particularly apparent upon contacting patients from the 2WW CNS MDT whereby the imaging findings are communicated via the patient's GP.

The included power calculation was based upon several assumptions; the extrapolation of effect size from an animal model of Alzheimer's disease to humans with cSVD; expected difference in signal to noise ratio between 3.0 and 7.0T; translation of a multi-flip angle multi-echo (MFAME) protocol to BBB-FEXI values and without specific consideration for inter-scanner variability. Whilst these are limitations, this hypothesis was generated based upon the expert consensus available at the time and we knew the main priority of the clinical WEX-BRAIN study was exploratory in nature and aimed to test the BBB-FEXI technique in a small group of participants with cSVD in line with the funding available.

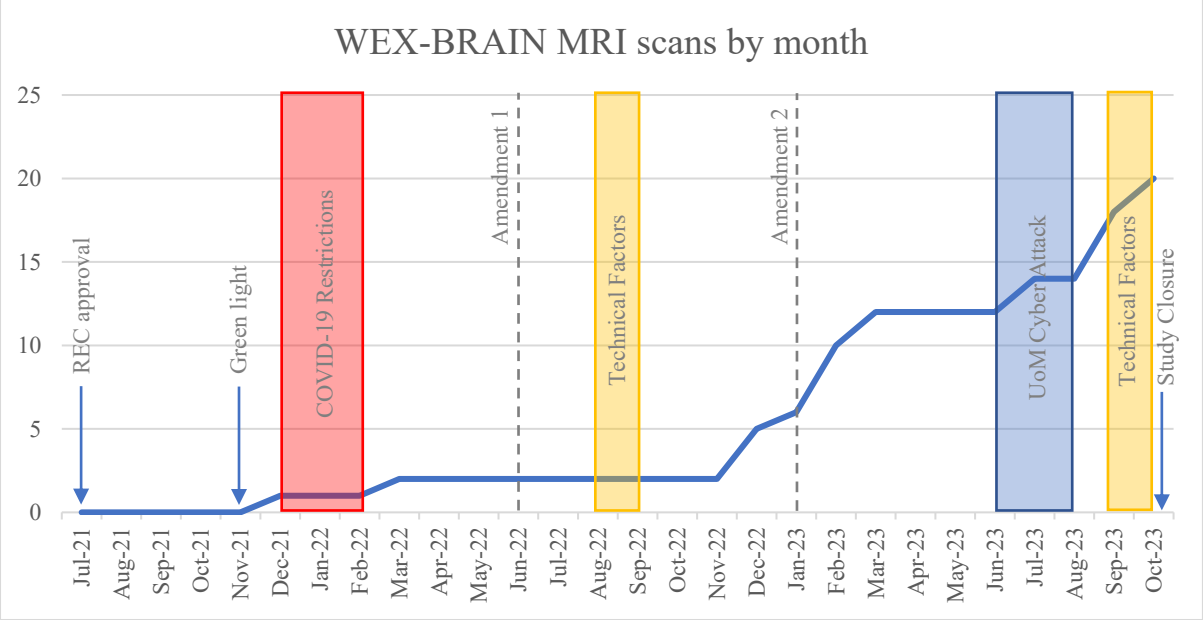


Figure 4-3: WEX-BRAIN completed MRI scans by month

I therefore arranged for a meeting of all relevant WEX-BRAIN stakeholders with a plan to submit a substantial ethics amendment to optimise recruitment (see Appendix 4-3).

Firstly, I set up a Participant Identification Centre (PIC) site at Salford Royal NHS Foundation Trust, with Dr Dwaipayan Sen as the local PI. Dr Sen provides a weekly tertiary cSVD clinic serving the North West of England from which participants could be prospectively recruited. I expected this addition would abrogate many problems relating to travel to the imaging site in Salford, given participants would already have undergone imaging at this location and would have attended the cSVD clinic on a face-to-face basis.

To address the concerns regarding home visits, I adjusted the requirements of Visit 1 so this could be offered as a telephone consultation, including the use of a telephone MoCA.[339] Several local imaging studies had adopted the use of bedside eGFR measurements performed on the day of imaging, prior to contrast administration, and I included the use of this technology within the amendment to streamline the healthy volunteer recruitment process if necessary.

Given the heterogeneity of the cSVD cohort and the priority for this exploratory study would be to recruit a range of cSVD patients – we relaxed our definition of 40 cSVD participants (20 with and 20 without a recent stroke) to be more flexible. Given the projected smaller number of overall recruitment, it was also decided that a single scanner (Salford Royal) would be utilised to exclude concerns regarding inter-scanner variability.

Despite this amendment, in summer 2022 I noticed that MRI scan appointments were limited. After discussion with the lead research radiographer at Salford Royal, this was deemed due to the backlog of scans amassed by several studies during the COVID-19 restrictions. A short

delay was also encountered due to necessary scanner firmware updates. I mitigated this by requesting imaging appointments many weeks in advance, but this required a lead-in time of several weeks before returning to actively scanning participants.

Through weekly attendance at the regional cSVD clinic, we identified that patients with moderate cSVD would often also have chronic kidney disease and therefore be ineligible for DCE-MRI scanning due to an eGFR <60 ml/min/1.73m^2. With retrospect, this was actually quite a novel insight at the time – though I note incidentally this trend was later reported separately by the CROMIS-2 study team (Cerebral microbleeds and intracranial haemorrhage risk in patients anticoagulated for atrial fibrillation after acute ischaemic stroke or transient ischaemic attack).[170] In line with the available literature and local practice, I submitted a minor amendment to reduce the eGFR threshold for contrast imaging to 40 ml/min/1.73m^2 (see Appendix 4-4).[313]

In October 2022, the difficulties with recruitment were discussed again with the wider WEX-BRAIN team. These were now mainly centred around the requirement for GBCAs and longer scan allocation times to facilitate DCE-MRI sequences. We noted that with scanner and clinician availability it was unlikely that we would meet our original recruitment target of 80 participants. We also did not envisage sufficient time for Visit 3 (follow up) to be completed during my allocated PhD time.

We therefore applied for a no-cost extension with the funding body (EPSRC) to allow for additional recruitment and scanning time, learning on 5th January 2023 that this had been extended to 17th November 2023. We also agreed that the priority for the WEX-BRAIN study

was to evaluate the novel BBB-FEXI technique. The decision was therefore made to forego the DCE-MRI sequence where necessary, alleviating the requirement for renal function testing.

Recruitment quickly gained pace and, despite some minor delays in summer 2023 in the context of disruption from a cyber-attack at University of Manchester, the requirement for further MRI scanner firmware updates and paternity leave, we were able to reach our updated recruitment target (10 cSVD, 10 healthy controls) completing our final scan on 24th October. To reach this target of 20 suitable scans, we had recruited a total of 26 participants – 3 cancelled multiple scanning appointments with short notice, 1 subject was unable to tolerate the scanning protocol due to claustrophobia, 1 had artefactual imaging and 1 scan was interrupted by technical factors (research *patch* failure). Insufficient DCE-MRI data was obtained for analysis.

The BBB-FEXI processing was performed by Dr Elizabeth Powell (University College London) given the expertise, software and hardware required to complete this. Initially, we had expected the BBB-FEXI sequence to result in an Apparent eXchange Rate (AXR) of water across the BBB. However, an interim publication by Dr Powell modelled further refinements in a series of simulations and evaluated these in healthy volunteers to show the impact of a 2 compartment model (intravascular and extravascular) and accounting for the T1 and T2 relaxation times of the respective tissue.[226] Overall, the 2 compartment model accounting for relaxation times (with water exchange measured as K_b*)* improved the accuracy whilst demonstrating acceptable scan-rescan reliability. We therefore selected K_b as the measure of choice, with values produced for white matter (WM), grey matter (GM) and WMHs.

Interestingly, their study found the mean K_b to be 2.95 s^{-1} and 2.27 s^{-1} in the WM and GM respectively. An initial review of our data revealed the mean K_b to be substantially higher at 14.40 s^{-1} in WM and 12.08 s^{-1} in GM. We acknowledged that this may be due to differences in acquisition parameters, inter-scanner differences or may be reflective of the lower age group imaged in the Powell *et al.* cohort. To investigate this, we recruited 2 further healthy volunteers (University of Manchester, study MHV003) with a mean age of 22.5 years (compared to a mean age of 62.5 year in the WEX-BRAIN cohort) and found the WM K_b 4.51 s^{-1} and GM K_b 3.29 s^{-1} more comparable, however, the overall association between K_b and age is less clear (see Figure 4-4).

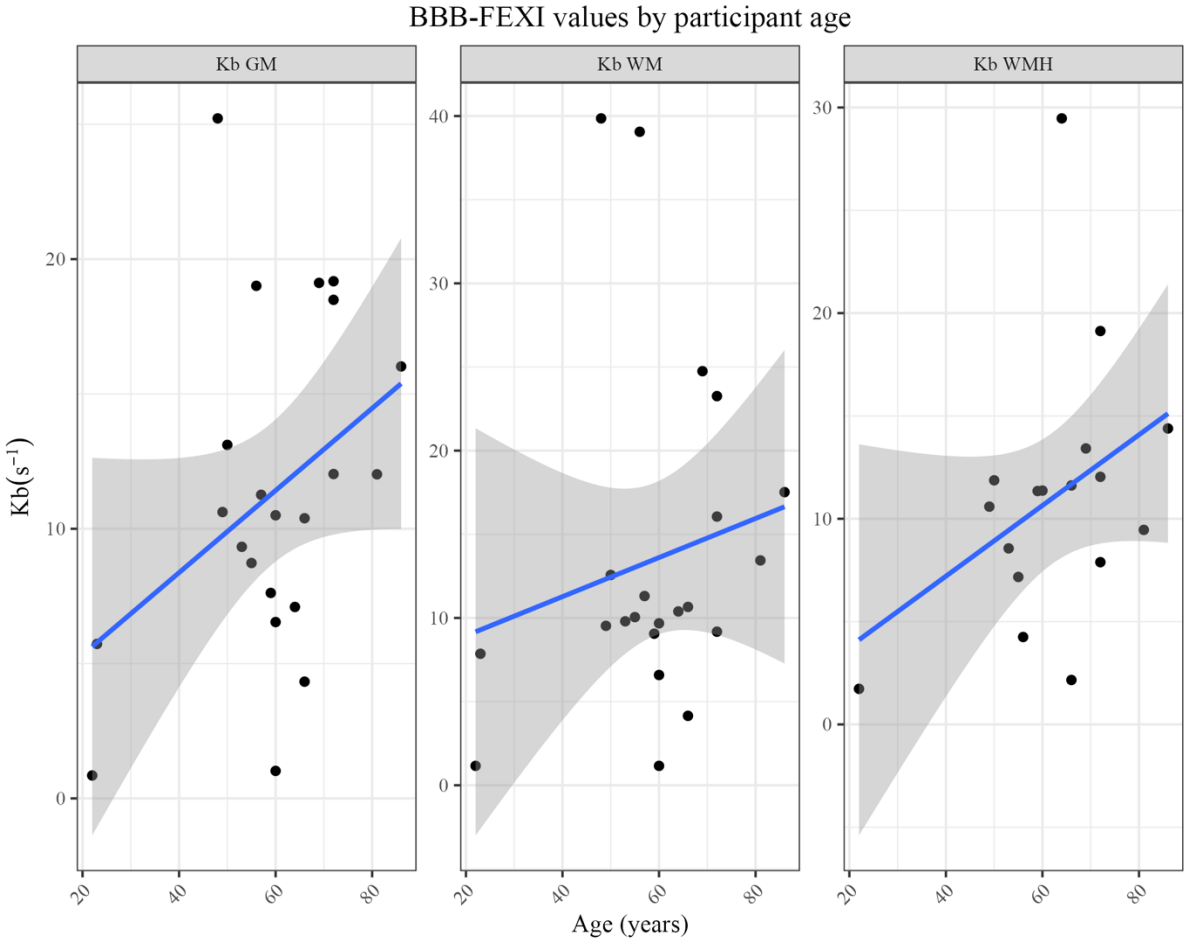


Figure 4-4: BBB-FEXI values in grey matter (GM), white matter (WM) and white matter hyperintensities (WMHs) according to age of participant at the time of scanning.

Derivation of K_b relies upon T1 / T2 values to determine the relaxivity of the underlying tissue. These values can be either be measured (or *mapped*) as part of the imaging protocol or substituted for literature values. For this analysis, we have selected to use measured values wherever possible - substituting literature values in the 4 participants where these were not available.

I have been trained to perform semi-automated analysis of WMHs using the Lesion Segmentation Toolbox in Matlab and volumetric analysis/segmentation of brain regions using Freesurfer (see Appendix 4-5 and 4-6). I considered utilising a *normalised* WMH volume for analysis – taking into account either brain or intracranial volume. This revealed a correlation value of 0.994 and 0.997 respectively, seen visually in Figure 4-5. This was due to the WMH volume varying by a factor of ~1000, compared to intracranial/brain volumes only varying by approximately 1.3. We therefore utilised an uncorrected WMH volume for the overall analysis.

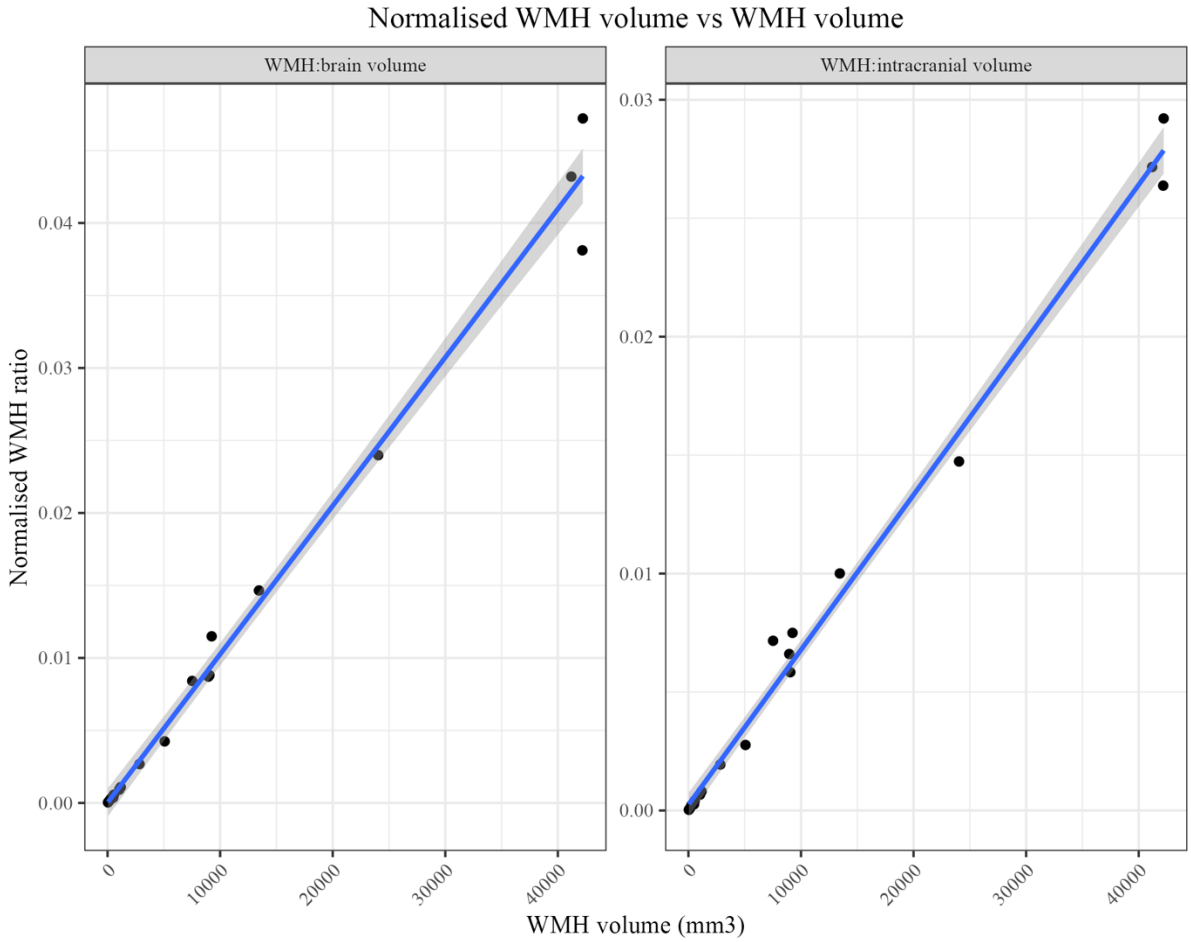


Figure 4-5: WMH volume when normalised to brain or intracranial volume.

The BBB-FEXI pre-processing and modelling will continue to be optimised, however, the initial outcomes are reported in Chapter 5.

Chapter 4 - Appendix List:

Appendix 4-1: Participant Information Sheet – cSVD

Appendix 4-2: Consent Form

Appendix 4-3: Amendment 1

Appendix 4-4: Amendment 2

Appendix 4-5: Protocol – cerebral volume measurement using Freesurfer

Appendix 4-6: Protocol – white matter hyperintensity measurement using LST in Matlab

Chapter 5: Filter-exchange imaging to evaluate for blood-brain barrier dysfunction in cerebral small vessel disease

Contribution statement:
This is the outcome of the clinical phase of the WEX-BRAIN study. I was responsible for analysing the clinical data, writing the draft manuscript and making all amendments.

I am grateful to both Elizabeth Powell (University College London) for completing the FEXI processing and providing the water exchange measurements and to Katie Moran (University of Manchester) for providing the summarised data from the online cognitive assessment.

Distribution:
The results of the WEX-BRAIN clinical study were analysed in May 2024 and remain under evaluation by the wider research team. We hope to publish a manuscript based upon these results in the near future.

Introduction:

The blood-brain barrier (BBB) separates the central nervous system (CNS) from the peripheral circulation. Composed of specialised brain endothelial cells, bound together by tight junctions and surrounded by neurones, astrocytes, pericytes, microglia and the basement membrane it forms the neurovascular unit.[63] Alongside the physical barrier function, shielding the CNS milieu from circulating toxins and immune cells, the BBB has essential roles in brain development, metabolism, angiogenesis, neurovascular coupling and immune regulation.[64,72]

Understanding of this complex interplay, even in health, is incomplete.[84] At present, the resolution of radiological studies is insufficient to directly image the BBB, animal models are not reflective of the environmental exposures, co-morbidities and vascular risk factors accumulated during a lifetime and post-mortem studies of brain tissue are neither representative of early-stage pathology – potentially more amendable to intervention – nor the dynamic and

multi-faceted interactions between BBB constituents. Nevertheless, changes in the BBB can be seen with normal ageing and accelerated BBB dysfunction has been implicated in a range of neurovascular, inflammatory and degenerative conditions.[90,100-102]

Perhaps in the simplest interpretation, BBB dysfunction can be considered as impairment to tight junctions resulting in unregulated paracellular extravasation (or *leakage*) of pathogens and toxins into the brain contributing to inflammation, gliosis and neuronal loss.[66,67] However, wider processes such as changes in active and passive transcellular transportation, impaired neurovascular coupling and deleterious immune cascades could all be contributory to alterations in metabolism, reduced waste clearance and neural degeneration.[88-92] How BBB dysfunction relates to the recently described glymphatic system and any downstream compensatory mechanisms still requires further consideration.[200] Nevertheless, there is growing interest in targeting BBB dysfunction as both a potential diagnostic and therapeutic marker.[128]

Evidence for subtle, diffuse BBB dysfunction as a core mechanism in the development of cerebral small vessel disease (cSVD) is increasing.[34,130,340-342] Presenting as an often overlooked, insidious clinical syndrome of cognitive impairment, mood disturbance and gait abnormalities, cSVD is responsible for approximately a quarter of all strokes and contributes to half of all cases of dementia.[7,12,231] However, the majority of cSVD is seemingly covert, identified *incidentally* on Magnetic Resonance Imaging (MRI) performed for other reasons and showing itself as white matter hyperintensities (WMHs) of presumed vascular origin, cerebral microbleeds, lacunes, enlarged perivascular spaces and brain atrophy.[37,38] The mainstay of consensus based management is based upon modification of vascular risk factors including smoking cessation, hypertension management and promotion of a healthy lifestyle.[60]

Radiological burden of WMHs has been shown to correlate with BBB function and measures of cognition.[131] However, at a patient level there may be little agreement between the clinical and radiological findings. This is, in part, due to a lack of sensitivity – with WMHs thought to only reveal the 'tip of the iceberg' compared to the wider microstructural changes – alongside a lack of specificity due to their additional links with demyelination, epilepsy, migraine and traumatic brain injury.[48,49,342]

The ability to non-invasively measure BBB dysfunction may present a more reliable marker of cSVD.[228,343-345] Given the availability of clinical hardware, relative cost and the potential for both high-resolution topographical and quantitative analysis without exposure to ionising radiation, MRI techniques have proved advantageous when compared to nuclear imaging, serum or CSF studies.[1,138,139,146,158,159] With increased diagnostic capabilities, there is scope for further understanding of BBB pathology and the potential to support clinical studies of therapeutics.

Measuring water exchange across the BBB using MRI has recently been proposed as a promising solution.[173,253] We know that water within the brain is compartmentalised, either in blood, cerebrospinal fluid, extracellular or intracellular fluid and movement between compartments is highly regulated. Indeed, there are potentially devastating consequences when this process is acutely disrupted.[176,178] Though the exact mechanisms of bi-directional BBB water exchange remain unclear, net flux is likely to be composed of simple diffusion, paracellular leakage and transcellular transportation.[174,179,180,186] Water can move against a concentration gradient, suggesting an active component to this process, with several co-transporters such as Na^+/K^+-ATPase and GLUT1 implicated. Aquaporins are water

transportation proteins, with aquaporin-4 being the most abundant subtype within the CNS, however these are predominantly located upon astrocyte endfeet on the abluminal side of the BBB - beyond the basement membrane.[189,190] Aquaporin-4 may therefore not be directly involved in the transportation of water across the BBB, but it may generate localised osmotic microgradients, alter BBB permeability to water via the release of chemical mediators and be involved with other brain-water homeostatic mechanisms such as the glymphatic system.[188,191-193] One hypothesis suggests that aquaporin-4 only assumes the rate limiting step of BBB water exchange in pathological, high-flux BBB states.[201]

At present, the majority of imaging studies measuring BBB water exchange are based upon Arterial Spin Labelling (ASL), a modality originally designed to measure tissue perfusion.[212] Blood water molecules are 'labelled' by inverting the longitudinal magnetisation of protons proximal to the imaging slice and, by obtaining a series of delayed images, the influx of labelled protons into this slice can be measured. The sensitivity of ASL to BBB water exchange is limited by the relatively small T1-weighted (T1w) difference between the intravascular and extravascular compartments resulting in low signal to noise ratio (SNR). This can be improved by the administration of contrast agents, utilising T2-weighted (T2w) imaging with multiple echo times or with diffusion-weighted ASL protocols.[215-217,226] Each of these solutions present their own limitations.

Filter exchange imaging (FEXI) is a novel, alternate MRI-based technique. FEXI was originally created to measure water exchange across membranes by exploiting the difference in diffusivity between compartments. FEXI has now been adapted to measure BBB water exchange (BBB-FEXI) by comparing the fast pseudo-diffusivity of intravascular water with the slow diffusivity of extravascular water as seen in Figure 5-1.

In principle, BBB-FEXI is composed of two pulse gradient spin echo (PGSE) blocks, separated by a longitudinal storage phase (mixing time).[224,225] The first PGSE block is the filter or 'crusher' gradient which aims to nullify any signal in the fast-diffusing, intravascular compartment. Water exchange then occurs during the mixing time and increases the apparent diffusion coefficient detected in the second, encoding PGSE block. By varying this mixing time, an ADC recovery curve can be measured. Fitting a simple one compartment model to the signal collected at a range of mixing times provides an estimation of the apparent exchange rate (AXR).

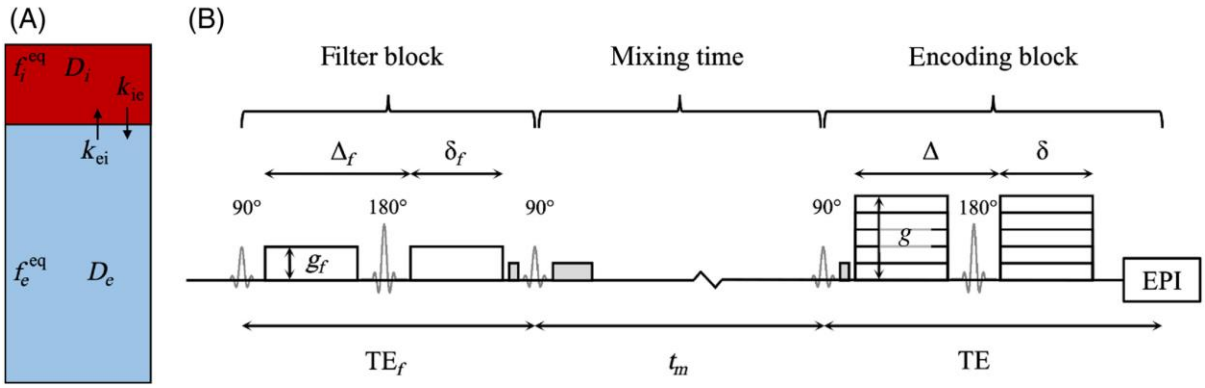


Figure 5-1: Filter exchange imaging. (A) The 2-compartment model, composed of intravascular (red) and extravascular (blue) tissue components subscripted *i* and *e*, respectively. Each compartment has an associated equilibrium signal fraction (f_i^{eq}, f_e^{eq}) and diffusivity (D_i, D_e) (note this is a pseudo-diffusivity in the intravascular compartment). (B) Pulse sequence diagram. The diffusion filter block (subscripted *f*) and encoding block are defined by the gradient strength (g_f, g), duration (δ_f, δ), and separation (Δ_f, Δ). Dephasing gradients before and after the longitudinal magnetisation storage pulses (second and third 90° pulses) and during the mixing time are shown in grey. Taken from Powell *et al.*[226] (with permission from Dr E Powell)

A recent study by Powell *et al.* demonstrated improved accuracy and good scan-rescan repeatability when utilising a 2-compartment model accounting for T1 and T2 relaxation times

to derive the BBB water exchange rate (K_b).[226] This addresses many of the limitations previously described.[253]

We utilise this refined BBB-FEXI technique for the first time in patients with cSVD, with comparison to healthy control participants (CPs), as part of an exploratory, hypothesis-generating study. Our primary outcome will be to detect a difference in K_b between cSVD and CP groups, with secondary outcomes examining for correlations between patient demographics, vascular risk factors and composite risk scores, conventional radiological markers (WMH volume) and measures of cognition.

Materials and Methods:

Participants:

This study was approved by Yorkshire and The Humber (Leeds West) Research Ethics Committee (21/YH/0119), the Health Research Authority and local research governance panels. Study sponsorship was provided by Lancashire Teaching Hospitals (LTH), with NIHR portfolio adoption and supported by the NIHR Lancashire Clinical Research Facility.

All participants provided informed written consent prior to enrolment. Participants with moderate/severe cSVD were prospectively recruited from 2 tertiary neurosciences centres in the North West of England (LTH and Northern Care Alliance NHS Foundation Trust), including directly from a tertiary cSVD clinic. Participants were identified by an experienced neurovascular neurologist or stroke physician with a specialist interest in cSVD, with input from a neuroradiologist if necessary. Screening, clinical evaluation and imaging supervision was completed by a neurology specialist trainee.

Eligibility criteria: Participants were required to be ≥18 years old, be fluent in English and to have undergone a recent MRI brain scan demonstrating moderate/severe cSVD (based upon visual assessment of conventional radiological markers – e.g., WMH burden considered to be Fazekas grade II/III).

Exclusion criteria: Participants were excluded if they had a contraindication to MRI scanning, a history of recreational drug use or a prior diagnosis of brain tumour, dementia, severe head trauma, multiple sclerosis or a neurodegenerative disorder.

Healthy volunteers of a similar age group were recruited for comparison, identified either through routine clinical practice, via university staff members or through our previous study database.

Clinical assessments:

All participants underwent a brief clinical assessment (see Chapter 4 for the full study protocol and Table 5-1 for a summary), medical questionnaire, measurement of baseline parameters (height, weight) and calculation of their BMI and QRISK3 score. After screening for depression, a cognitive evaluation included the Montreal Cognitive Assessment (MoCA), Brief Memory and Executive Test (BMET) and a University of Manchester (UoM) online cognitive battery (completion time ~10 mins) including basic recognition, semantic, visual and motor tasks.

Tests were performed in a fixed order to ensure standardisation. The entire assessment was designed to last a total of approximately 1 hour.

	Visit 1	Visit 2	Visit 3
Medical questionnaire	**X**		**X**
MRI safety questionnaire	**X**	**X**	
BMI	**X**		**X**
QRISK3	**X**		**X**
PHQ-2/PHQ-9	**X†**		**X†**
Edinburgh Handedness Inventory		**X**	
MRI scan (BBB-FEXI)		**X**	
MoCA	**X**		**X**
BMET	**X**		**X**
UoM online cognitive battery	**X**		**X**

Table 5-1: Study assessment schedule. †PHQ-9 will only be completed if the PHQ-2 score is ≥ 3.

MRI scanning:

All scans were performed at Salford Royal Hospital using a Philips 3.0 T MRI scanner, with a 32-channel head coil.

Structural Imaging:

Structural imaging sequences were obtained for registration and segmentation purposes, including T1w, T2w, fluid-attenuated inversion recovery (FLAIR) and susceptibility-weighted imaging (SWI).

Filter exchange imaging (BBB-FEXI):

BBB-FEXI data was acquired using an in-house developed encoding sequence (see Table 5-2 for parameters).

	BBB-FEXI			
Resolution (mm^3)	3 x 3 x 5			
Repetition time, TR (ms)	5000			
Echo time, TE (ms)	63			
***b*-values (s/mm^2)**	0, 50, 100, 250, 1000			
Gradient directions	6, 6, 6, 6, 6			
Averages	1			
Total volumes	120			
Scan time	16 minutes 5 seconds			
Filter echo time, TE_f (ms)	42			
Filter *b*-values, b_f (s/mm^2)	0	250	250	250
Mixing time, t_m (ms)	16	16	200	400

Table 5-2: FEXI imaging acquisition parameters

Image analysis:

MRI data was transferred via locally agreed protocols to the University of Manchester and Lancaster University for analysis. All imaging was reviewed for significant incidental findings by a consultant neuroradiologist. Structural imaging was analysed to measure intracranial, whole brain, region of interest, white matter and grey matter volume by established methods with Freesurfer version 7.3.2.[346] WMH number and volume was measured as a marker of cSVD using Lesion Segmentation Toolbox using both T1w and FLAIR sequences in SPM12 for MATLAB R2022b with a locally established threshold of 0.3.[101,347]

FEXI processing:

Regional brain BBB-FEXI imaging was processed using a two-compartment model in MATLAB as previously reported by Powell *et al* to calculate the BBB water exchange rate (K_b).[226] Where available, T1/T2 maps were utilised to account for tissue relaxation rates, otherwise standard literature values were substituted. Before fitting, signals were normalised using the signal at $b = 0$ (in the encoding block) with corresponding filter *b*-value and mixing

time. Equilibrium blood signal fractions were fixed at 5% in the grey matter (GM) and 3% in the WM for the compartmental models to stabilise fitting.[348-350] Free parameters in the model were intra- and extravascular diffusivity (D_i and D_e respectively) and K_b. Parameters were constrained to 0.1 $um^2/ms \leq D_e \leq 3.5$ um^2/ms, 3 $um^2/ms \leq D_i \leq 100$ um^2/ms and $K_b > 0$ s^{-1}. Visual parameter maps were generated for D_i, D_e and K_b using the median value for each region of interest (ROI).

The full BBB-FEXI pre-processing methodology and literature values for T1/T2 maps can be found in Appendix 5-1.

Statistical methods:

Participants were grouped into those with cSVD and CPs. Participant demographics, co-morbidities, vascular risk factors and performance scores were compared between groups. Given the small group size and data distribution, non-parametric tests were selected. Fisher's exact test was utilised to compare categorical variables and Mann-Whitney U tests to examine continuous variables. Averages, where reported, are presented as the mean value ± standard deviation.

Recruitment of cSVD participants was based upon prior analysis of routinely performed imaging (i.e., using a visual rating scale). We therefore expected to find a difference in the number and volume of WMHs derived from the Lesion Segmentation Toolbox between groups and utilised an unpaired Mann-Whitney U test to confirm this.

To address our primary outcome, we performed paired and unpaired Mann-Whitney U tests to compare K_b (with both measured and literature T1/T2 values), as well as for D_i and D_e. We

calculated Pearson's correlation coefficients for K_b and D_e in GM, WM and WMH with age, QRISK3 and WMH volume to assess for correlation between novel imaging markers and both vascular risk factors and cSVD burden on conventional imaging. We did not examine for correlation with D_i as this represents the pseudo-diffusivity of intravascular water which, though may differ slightly due to changes in cerebral blood flow, is unlikely to reflect the endothelial/parenchymal change expected in cSVD and there was no difference found in D_i between groups during preliminary data analysis.

Cognitive scores are compared between groups using Mann-Whitney U tests and with imaging measures (WMH volume and K_b) using Pearson's correlation coefficients. MoCA scores are presented as an overall value (maximum score 30) as well as the constituent domains of visuospatial, naming, attention, language, abstraction, delayed recall and orientation. BMET scores (maximum score 16) are presented as an overall value alongside executive and memory subsets. The UoM online cognitive battery was analysed to produce mean response time (number of questions completed divided by the time taken) and accuracy (correct answers divided by total questions answered).

Statistical analysis was completed in R v4.1.3.[243]

Results:

A total of 25 participants were recruited, 14 with moderate/severe cSVD and 11 control participants (CP). Complete imaging data for 5 subjects was not obtained (2 due to cancellation of scanning visits, 1 subject was unable to tolerate the scanning protocol, 1 was noted to have artefactual images and 1 had a scan interrupted by technical factors). We therefore include a

total 10 cSVD and 10 CPs for the main analysis. In 4 cases, T1/T2 maps were not available and literature values were substituted. Summary demographics can be found in Table 5-3.

	Controls	**cSVD**	*p*
No. of participants	10	10	
Age	56.2 ± 5.1	69.3 ± 10.2	0.004**
Sex	M: 3 F: 7	M: 4 F: 6	
Hypertension	0	8	0.0007***
Ischaemic heart disease	0	2	0.47
Heart failure	0	1	
Stroke/TIA	0	3	0.21
Migraine	1	6	0.057
Diabetes mellitus	0	2	0.47
Hyperlipidaemia	0	4	0.087
Smoking pack years	2.7 ± 4.8	10.4 ± 13.3	0.25
Non-smoker	7	5	
Ex-smoker	3	4	
Current smoker	0	1	
Alcohol units/week	6.0 ± 6.3	3.3 ± 4.8	0.33
Body mass index	26.1 ± 4.42	29.4 ± 6.59	0.44
QRISK3 score	5.71 ± 3.35	23.8 ± 13.5	0.00002***
WHO performance score			
0	10	2	
1	0	3	
2	0	5	
Mean number of medications	1.3	5.6	0.0006***
≤ 2 medications	10	0	
> 2 medications	0	10	

Table 5-3: Participant demographics. Abbreviations: cSVD: cerebral small vessel disease, TIA: Transient Ischaemic Attack. Note: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Control participants are noted to be younger than the cSVD group (mean age 56.2 vs 69.3 years, $p=0.004$) and, as expected, had a lower QRISK3 score, were less likely to be

hypertensive or be prescribed multiple medications (p<0.001 for each). A trend towards a higher prevalence of migraine (p=0.06) and hyperlipidaemia (p=0.09) in the cSVD group almost reached significance (see Figure 5-2).

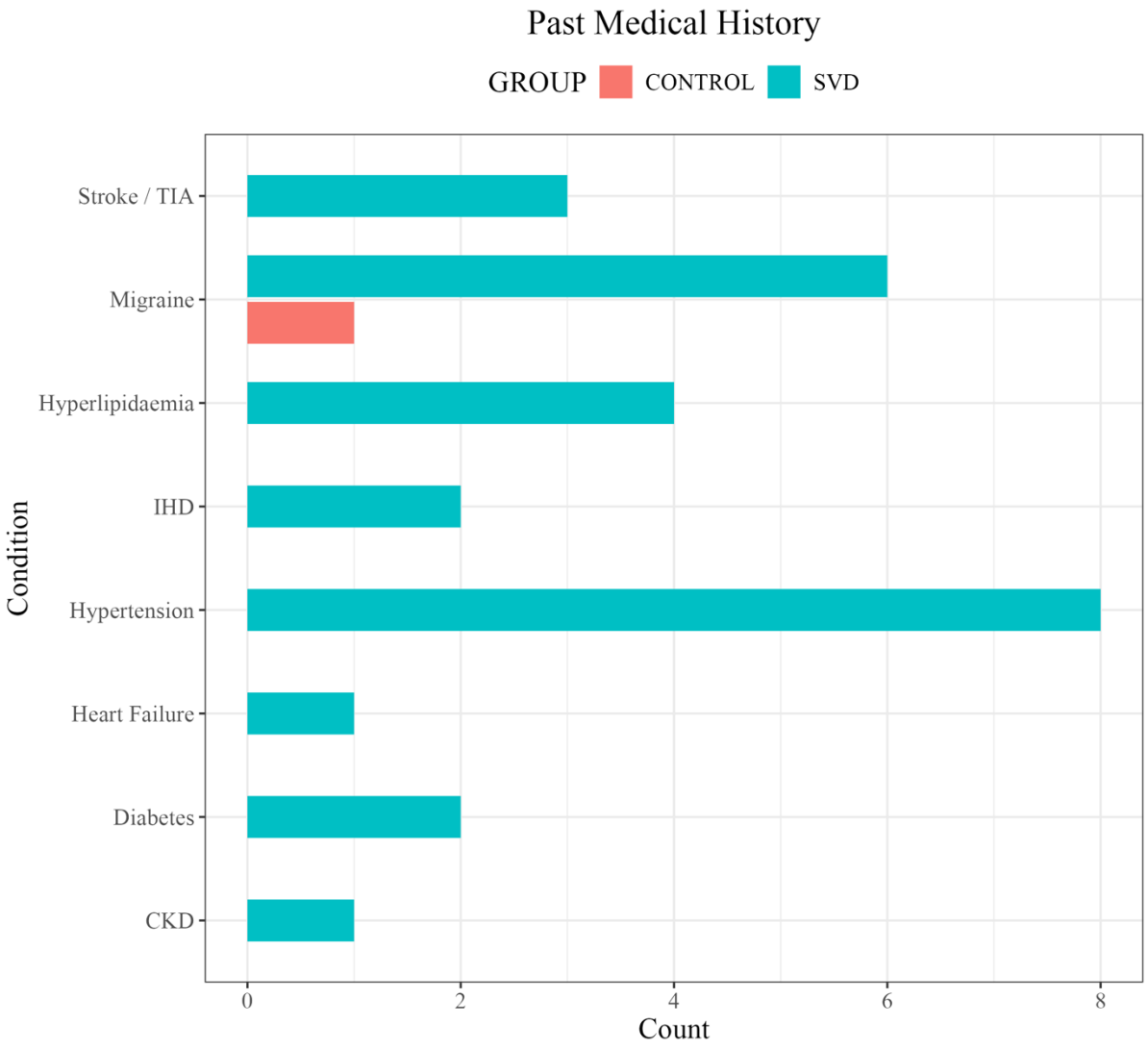


Figure 5-2: Past medical history of participants Abbreviations: TIA: Transient Ischaemic Attack, IHD: Ischaemic Heart Disease, CKD: Chronic Kidney Disease.

Imaging analysis:

Structural imaging:

As seen in Table 5-4, cSVD participants had a significantly higher mean volume of WMHs at 20292 ± 15738 mm^3 compared to CPs at 738 ± 819 mm^3 (p<0.001). The total number of WMHs was also higher in the cSVD group (p<0.001). This was expected given the recruitment method.

	CP	**cSVD**	*p*
Conventional WMH analysis			
N. WMHs	6.1 ± 3.3	23.1 ± 9.50	0.0004***
WMH volume (mm^3)	738 ± 819	20292 ± 15738	0.00001***
Diffusivity measurements:			
GM D_i (um^2/ms)	16.8 ± 5.83	20.0 ± 10.8	0.74
WM D_i (um^2/ms)	17.7 ± 8.42	18.7 ± 11.79	0.97
WMH D_i (um^2/ms)	13.9 ± 3.13	18.0 ± 11.9	0.56
GM D_e (um^2/ms)	0.848 ± 0.0711	1.01 ± 0.128	0.002**
WM D_e (um^2/ms)	0.661 ± 0.0530	0.771 ± 0.0624	0.002**
WMH D_e (um^2/ms)	1.11 ± 0.372	1.04 ± 0.135	0.87
Water exchange (BBB-FEXI) analysis:			
GM K_b (s^{-1})	10.9 ± 6.74	13.2 ± 5.02	0.22
WM K_b (s^{-1})	14.7 ± 13.3	14.1 ± 6.46	0.48
WMH K_b (s^{-1})	11.9 ± 8.97	11.3 ± 4.41	0.37

Table 5-4: Imaging measurements between groups. Abbreviations: cSVD: cerebral small vessel disease, TIA: Transient Ischaemic Attack. Note: * p<0.05, ** p<0.01, *** p<0.001.

Filter-exchange imaging:

Visual parameter maps and corresponding FLAIR imaging from a representative CP and cSVD subject are shown in Figure 5-4 and 5-5 respectively. No significant difference is seen between K_b in WM or WMHs between cSVD and CP groups (p=0.48, p=0.37 respectively). We repeated the analysis using only literature values for the T1/T2 maps and also found no significant difference between K_b in WM (p=0.35) or WMH (p=0.79) between groups. Whilst

K_b appeared lower within WMHs when compared to WM, this did not reach statistical significance with a paired analysis of all participants (p=0.22). An overall comparison between WM and WMH K_b using our measured and substituted literature T1/T2 values can be seen in Figure 5-6. Whilst GM K_b appeared higher in the cSVD group (13.2 ± 5.02 compared with 10.9 ± 6.74 s^{-1} in CPs) this also did not reach significance (p=0.22).

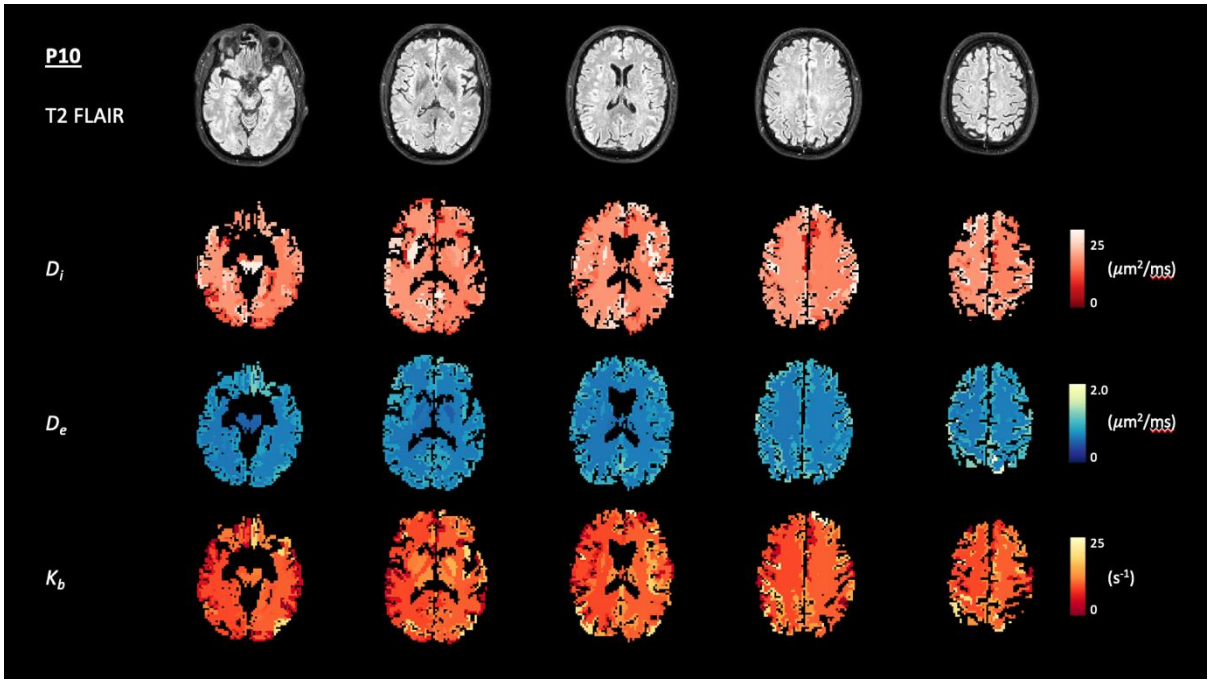


Figure 5-4: FLAIR imaging and parameter maps for a control participant. All maps display the median value within each ROI; both extreme fit values and masked CSF are shown in black.

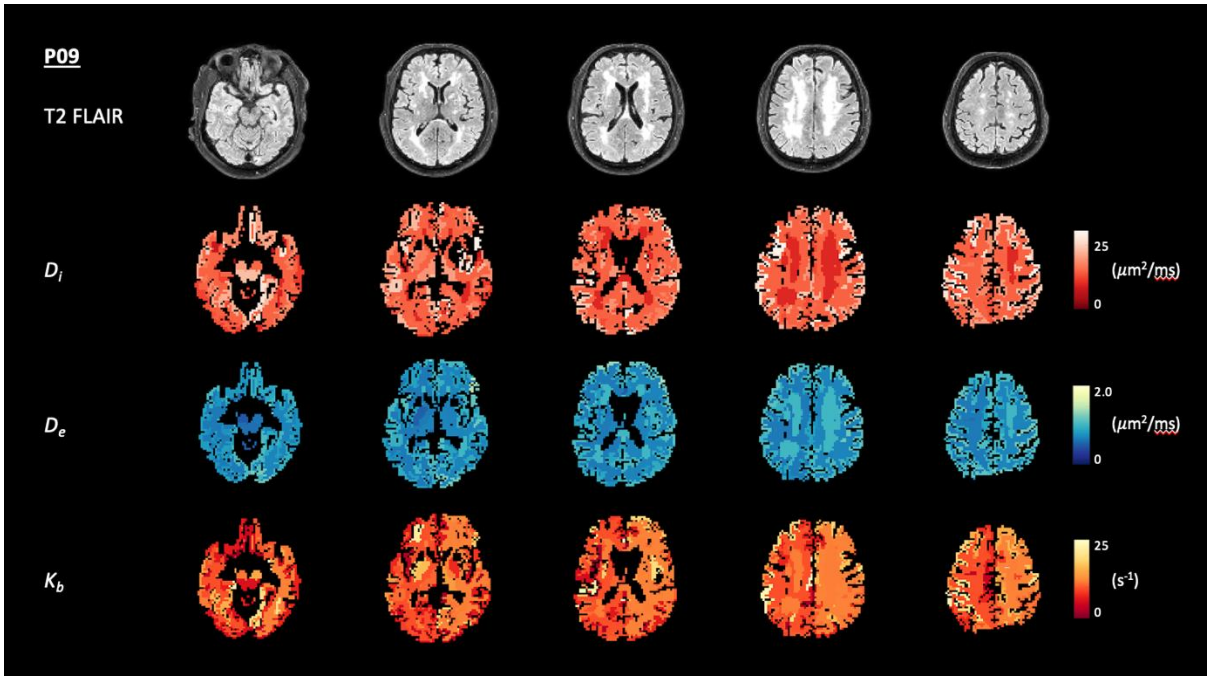


Figure 5-5: FLAIR imaging and parameter maps for a cSVD participant. All maps display the median value within each ROI; both extreme fit values and masked CSF are shown in black.

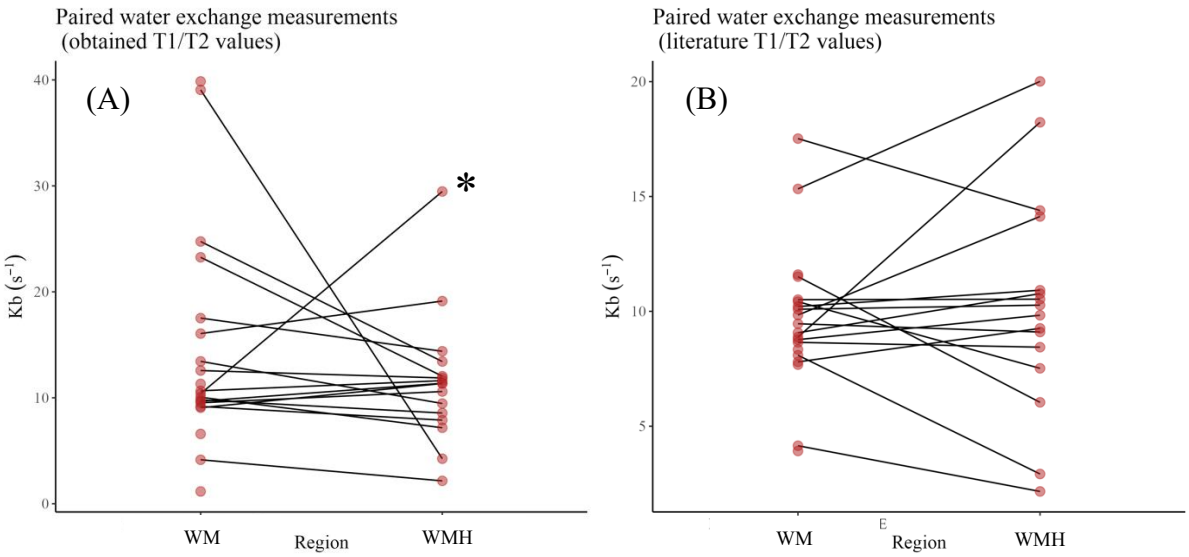


Figure 5-6: Paired water exchange rate (K_b) in WM and WMHs as seen in (A) the main analysis, using T1/T2 values as measured and (B) where only literature values are used. *Outlier represents participant 30, a healthy volunteer.

When considering measurements of diffusivity, we found that D_e was significantly higher in the cSVD group in GM (p<0.01) and WM (p<0.01), but interestingly not within WMHs (p=0.87). Performing a paired analysis between WM and WMHs including all participants revealed a significant difference in D_e (p<0.001).

There was no difference, as expected, in D_i between cSVD and CPs in GM, WM or WMH (p=0.74, p=0.97 and p=0.56 respectively).

Imaging comparisons:

Strong positive correlation was seen between WMH volume and age (see Figure 5-3, r=0.668, p<0.01) as has previously been reported.[47] However, the Pearson's correlation coefficient was fractionally higher at r=0.683 (p<0.01) for WMH volume and QRISK3 score. We note that QRISK3 score is significantly influenced by age, but also accounts for several additional vascular risk factors such as smoking and diabetes status, family history of cardiovascular disease and measurement of systolic blood pressure, height and weight. Further work is required to understand whether the additional components included in the QRISK3 calculation can better describe WMHs than age alone.

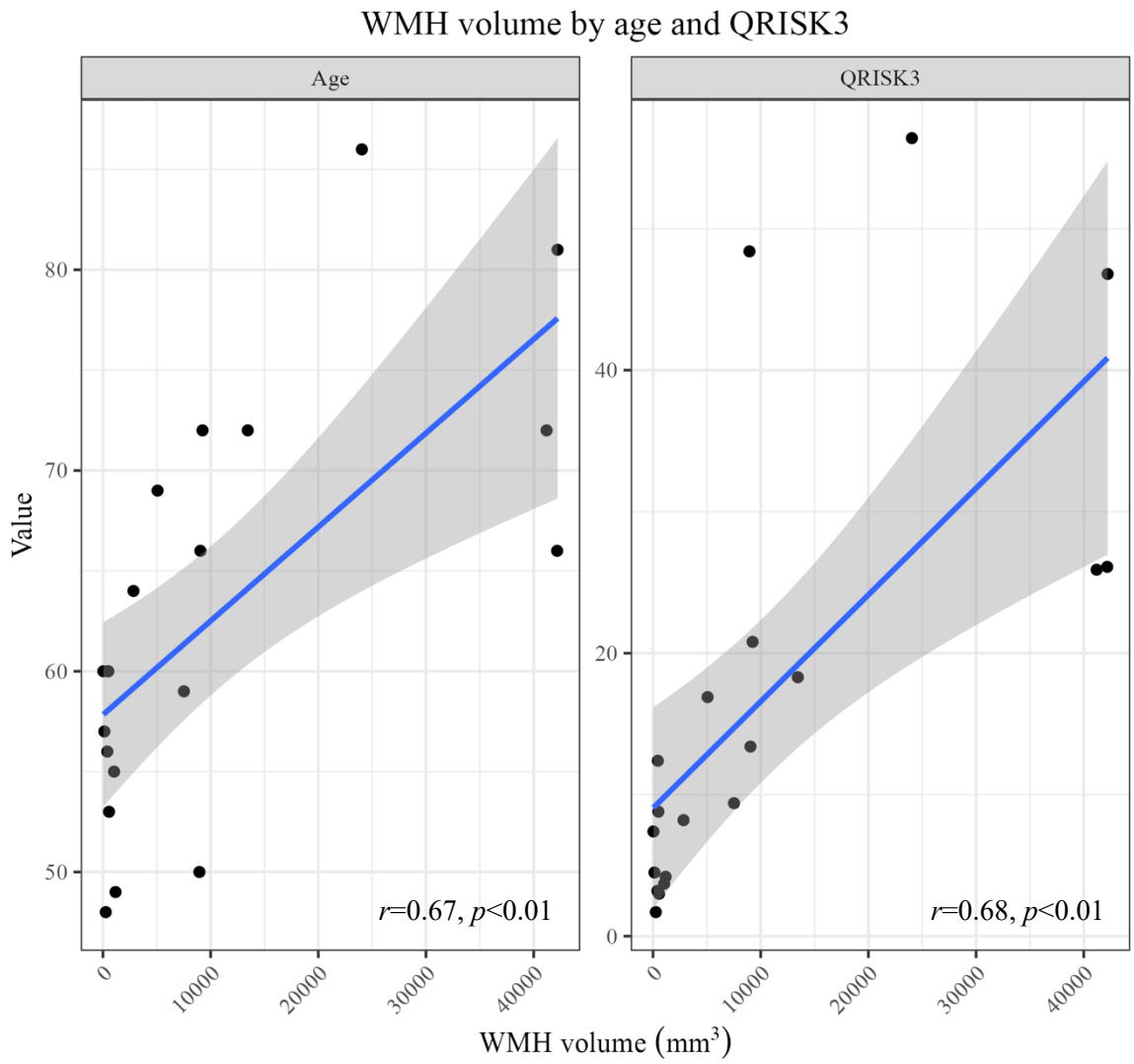


Figure 5-3: WMH volume correlated with age and QRISK3 score

As seen in Table 5-5, there was no significant correlation between K_b in GM, WM or WMH and either age or QRISK3 score. Similarly, as seen in Figure 5-7, there was no significant correlation between K_b values and WMH volume (r= -0.086, p=0.75).

	Age	QRISK3	WMH volume
GM D_e	r=0.76, p=0.000097***	r=0.68, p=0.0011**	r=0.58, p=0.0070**
WM D_e	r=0.73, p=0.00028***	r=0.63, p=0.0029**	r=0.64, p=0.0025**
WMH D_e	r=0.040, p=0.88	r= -0.14, p=0.61	r=0.070, p=0.80
GM K_b	r=0.097, p=0.69	r=0.16, p=0.51	r=0.056, p=0.81
WM K_b	r= -0.073, p=0.76	r= -0.066, p=0.78	r= -0.11, p=0.64
WMH K_b	r=0.20, p=0.47	r=0.093, p=0.73	r= -0.086, p=0.75

Table 5-5: Pearson's correlation co-efficient table for age, QRISK3 and imaging parameters. Abbreviations: GM: grey matter, WM: white matter, WMH: white matter hyperintensity, D_e: Extravascular diffusivity, K_b: BBB water exchange. Note: * p<0.05, ** p<0.01, *** p<0.001.

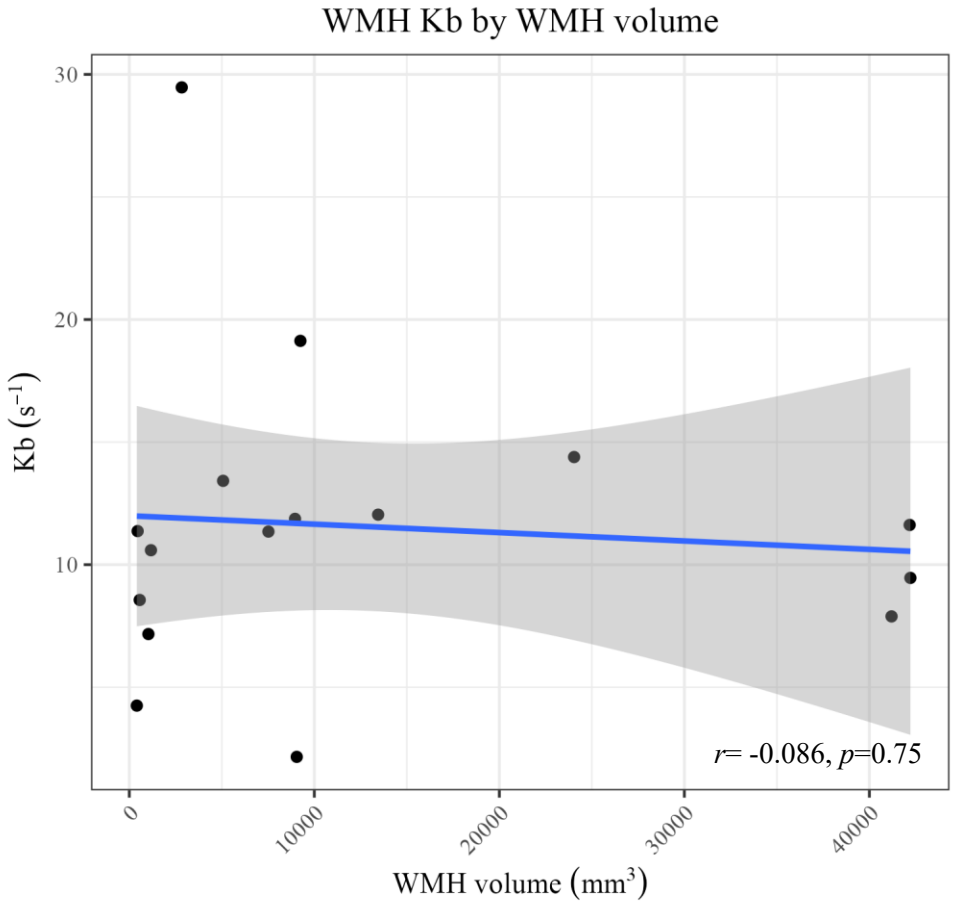


Figure 5-7: WMH K_b and WMH volume.

However, there was a significant positive correlation between D_e in both GM and WM with participant age and QRISK3. The correlation coefficients appear very similar to those seen

between WMH volume and both age and QRISK3. D_e values were positively correlated with WMH volume in both GM (r=0.58, p=0.0070) and WM (r=0.64, p=0.0025).

Cognitive Analysis:

Overall MoCA score was higher in CPs compared to the cSVD group (p<0.001), with the attention (p<0.05) and delayed recall (p<0.001) subsections reaching significance (see Table 5-6). A MoCA result of ≥26 is considered a normal result, with the 5/10 cSVD participants scoring below this threshold indicative of cognitive impairment. Similarly, average BMET scores were higher by 3.2 points in the CP group (p<0.05) with a greater difference seen in the executive subset (p<0.05) than the memory subset (p<0.05).

	CP	cSVD	*p*
MoCA overall:	29.4 ± 0.84	24.3 ± 4.4	0.0005***
Visuospatial	4.6	3.9	0.16
Naming	3	2.9	0.37
Attention	6	5.3	0.035**
Language	2.9	2.6	0.28
Abstraction	2	1.8	0.17
Delayed recall	4.9	2.1	0.0003***
Orientation	6	5.7	0.17
BMET overall:	15.9 ± 0.32	12.7 ± 4.5	0.019*
Executive	7.9	6.1	0.019*
Memory	8	6.6	0.035*
UoM online cognitive battery:			
Basic Recognition Task			
Response time (ms)	447 ± 77.7	607 ± 154	0.006*
Accuracy	0.993 ± 0.01	0.985 ± 0.03	0.84
Semantic Task:			
Response time (ms)	1722 ± 434	2567 ± 1432	0.20
Accuracy	0.979 ± 0.017	0.953 ± 0.06	0.56
Motor Task			
Response time (ms)	1146 ± 177	1718 ± 838	0.043*
Accuracy	1 ± 0	1 ± 0	n/a
Visual Task			
Response time (ms)	2171 ± 448	2263 ± 524	0.76
Accuracy	0.888 ± 0.067	0.770 ± 0.135	0.033*

Table 5-6: Cognitive profiles by group. Abbreviations: MoCA: Montreal Cognitive Assessment, BMET: Brief Memory and Executive Test, UoM: University of Manchester. Note: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

The exploratory UoM online cognitive battery of basic recognition, semantic, motor and visual tasks were scored by the average response time to each question and the accuracy of answers. The CP group answered questions in a shorter duration of time (see Figure 5-8) in the basic recognition ($p<0.01$) and motor tasks ($p<0.05$) with an average reduction of 160ms and 572ms respectively. The variability between groups is also different, with a broader range of response

times for cSVD participants in the basic recognition, semantic and motor tasks. Accuracy was significantly different in the visual task (p<0.05).

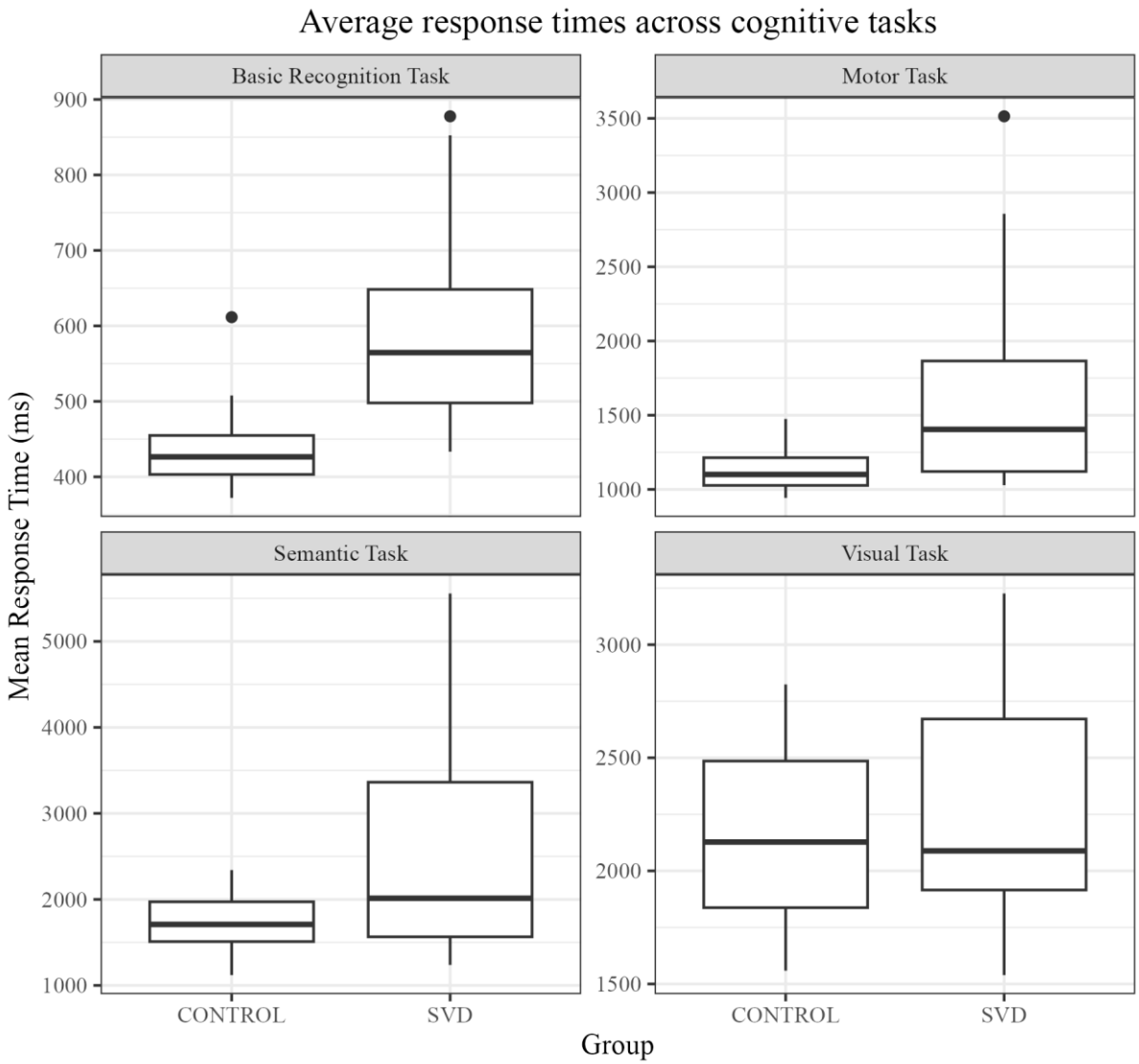


Figure 5-8: Average response times across cognitive tasks

There is clear negative correlation between WMH volume and MoCA overall score, seen most clearly within the subsections of attention, delayed recall and visuospatial/executive function (see Figure 5-9, Table 5-7). This can also be seen with the total BMET score and both subsections (see Figure 5-10), though as expected the correlation is stronger with the executive subset. Similarly, increasing WMH volume was associated with increased response time in the

UoM online cognitive battery (see Figure 5-11). No significant correlation was seen between K_b and any of the cognitive measures tested.

	WMH volume	**WMH K_b**
MoCA total	r= -0.83, p=0.0000053***	r=0.11, p=0.69
Abstraction	r= -0.34, p=0.15	r=0.10, p=0.70
Attention	r= -0.74, p=0.00020***	r=0.16, p=0.54
Delayed recall	r= -0.71, p=0.00048***	r=0.13, p=0.62
Language	r= -0.51, p=0.021*	r=0.093, p=0.73
Naming	r= -0.49, p=0.029*	r=0.16, p=0.56
Orientation	r= -0.55, p=0.013*	r=0.088, p=0.75
Visuospatial / Executive	r= -0.72, p=0.00035***	r= -0.12, p=0.66
BMET total	r= -0.79, p=0.000034***	r=0.097, p=0.72
Memory	r= -0.72, p=0.00036***	r=0.067, p=0.81
Executive	r= -0.84, p=0.0000040***	r=0.12, p=0.65
UoM online cognitive battery:		
Basic Recognition Task		
Response Time	r=0.77, p=0.00019***	r= -0.15, p=0.61
Accuracy	r= -0.36, p=0.14	r=0.16, p=0.59
Motor Task		
Response Time	r=0.74, p=0.00048***	r= -0.15, p=0.62
Accuracy	n/a	n/a
Semantic Task		
Response Time	r=0.62, p=0.0060**	r=0.090, p=0.76
Accuracy	r= -0.69, p=0.0014**	r=0.20, p=0.49
Visual Task		
Response Time	r=0.38, p=0.12	r=0.25, p=0.38
Accuracy	r= -0.78, p=0.00012***	r=0.40, p=0.15

Table 5-7: Pearson's correlation coefficient table for cognition and imaging parameters. Abbreviations: WMH: White Matter Hyperintensity, K_b: BBB water exchange, MoCA: Montreal Cognitive Assessment, BMET: Brief Memory and Executive Task, UoM: University of Manchester. Note: * p<0.05, ** p<0.01, *** p<0.001.

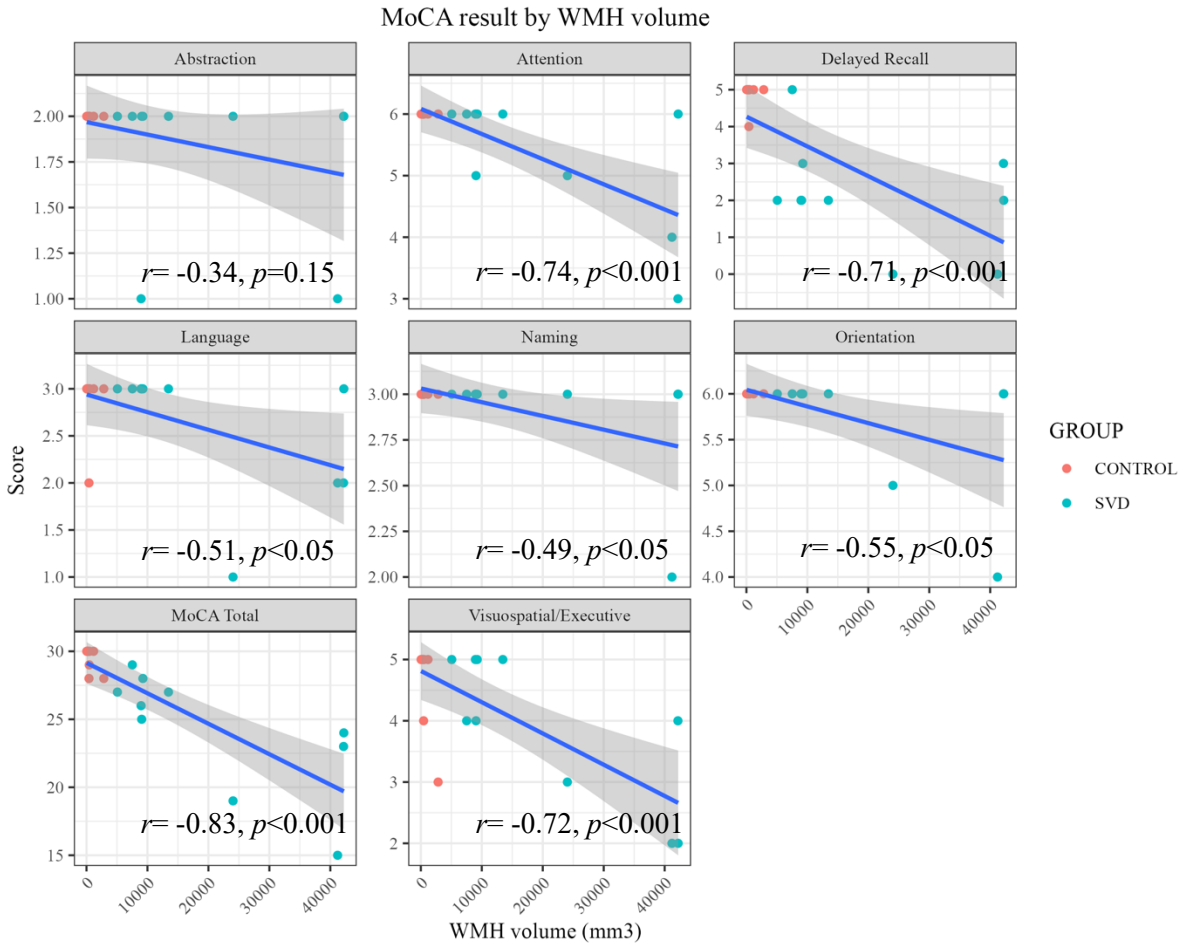


Figure 5-9: MoCA scores are associated with WMH volume

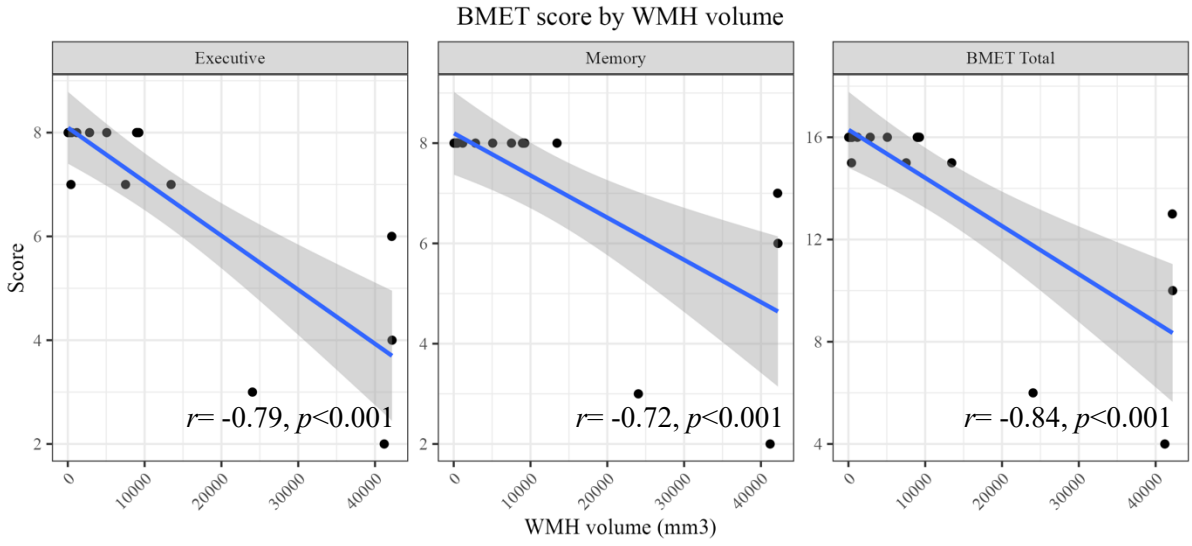


Figure 5-10: BMET scores are associated with WMH volume

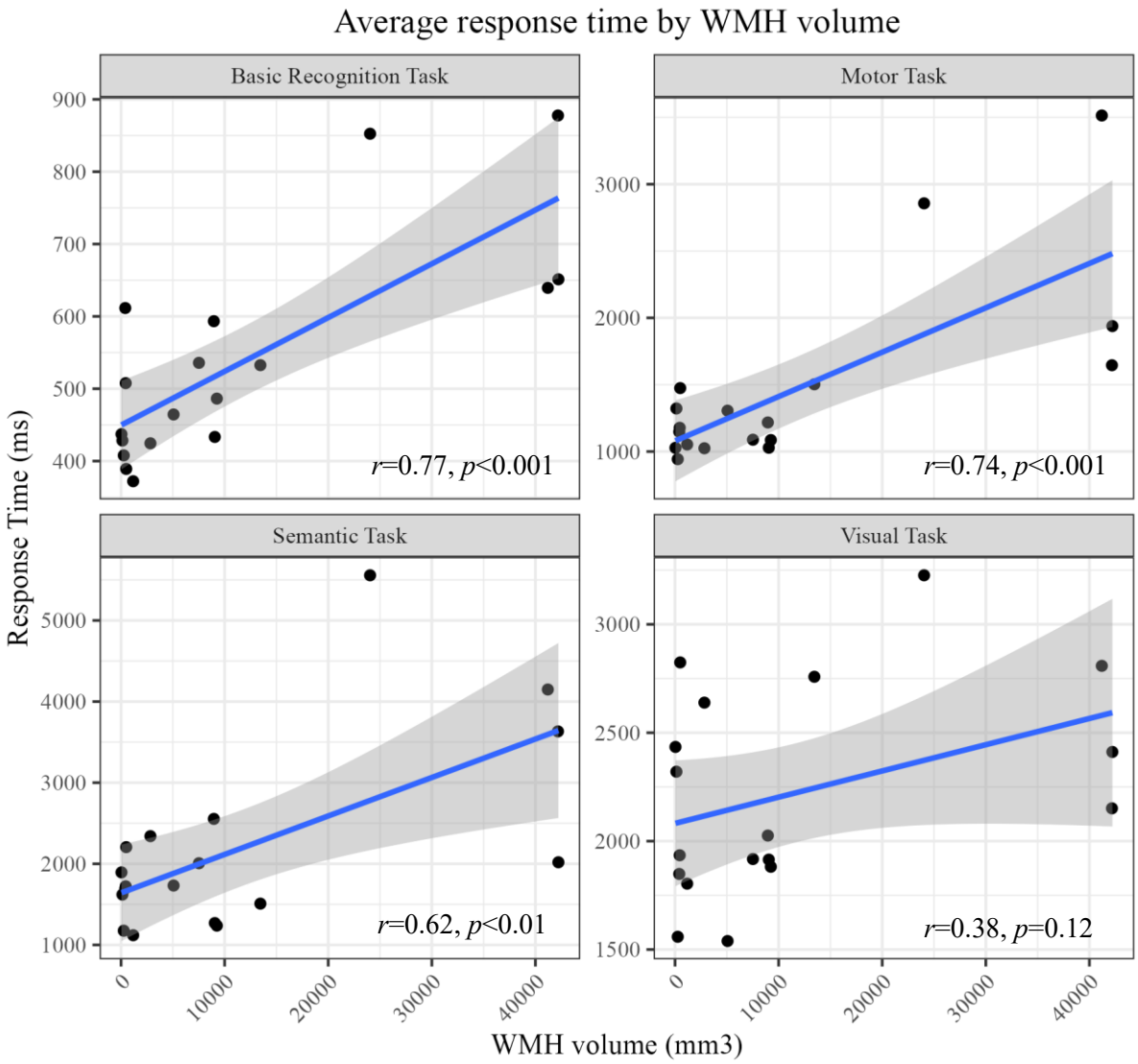


Figure 5-11: Response times (indicative of processing speed) measured by the UoM online cognitive battery are associated with WMH volume

Discussion:

In this exploratory analysis, we did not find a difference in K_b between groups within GM, WM or WMHs. K_b appeared lower in WMHs compared with WM in both groups, but this was not statistically significant. We suspect this may reflect the study being underpowered due to the small sample size, however the relatively heterogeneous cohort in terms of age and comorbidity may be contributory to the high variability in K_b measurements.

Nevertheless, our exploratory study has several interesting findings. Firstly, it is possible to perform BBB-FEXI measurements using only clinically available hardware and the imaging protocol is well tolerated by participants with cSVD. Only 1 participant (<5%) was unable to complete the full imaging protocol, comparable to the rate expected in clinical practice.[330]

We report a possible relationship between QRISK3 and WMH volume. This requires further validation, particularly to separate out the effect of age, but highlights the importance of WMHs as an early warning signal for cardiovascular events and indicates the need to further evaluate such patients for the presence of modifiable risk factors such as hypertension.

Perhaps the most compelling findings were related to the extravascular diffusivity measurements calculated from diffusion weighted imaging (DWI) sequences. As well as significant correlations between D_e and both age and QRISK3, we demonstrated a difference in GM and WM D_e between groups. Measures of diffusivity could be identifying changes in the normal appearing brain parenchyma which are not yet manifesting with WMHs. Indeed, we have reported a significant correlation between WMH volume and D_e, further supporting the hypothesis that cSVD is a diffuse process. D_e is regarded as a measure of microvascular structural integrity, similar to Mean Diffusivity (MD) measured with Diffusion Tensor Imaging (DTI). Several DTI studies have reported correlations between MD and fractional anisotropy (FA) with disease severity and cognitive performance in cSVD.[351,352] As part of our exploratory imaging protocol, we had also captured DTI sequences and (from personal communication with Dr Elizabeth Powell) there is strong correlation between D_e and MD in the WM of all participants (r=0.78, p<0.001). Further, whilst we did not find any difference in D_e for WMHs between groups, nor was there a difference in FA or MD between groups (p=0.62 and p=0.13 respectively), there was significant intra-individual difference in WM and WMH

in a paired analysis (D_e p<0.001, FA p<0.05, MD p<0.001). This suggests that the changes in structural integrity within WMHs is similar despite the burden of cSVD. Longitudinal studies, with comparison to clinical measures, conventional radiological and DTI sequences, would be required to understand whether regional D_e measurements are a sensitive measure of cSVD severity and if these changes could pre-date the appearance of WMHs or correlate with clinical manifestations such as cognitive decline.

We note that the K_b values reported in GM, WM and WMHs for the cSVD and CP groups are higher than those previously reported in the literature. An identical imaging protocol was completed on the same scanner as part of a separate study (University of Manchester: MHV003) in 2 younger healthy volunteers (22 and 23 years old) in whom water exchange rate was lower in GM (K_b = 3.29 s^{-1}, p<0.05) and WM (K_b = 4.51 s^{-1}, p=0.07) and appeared in keeping with those by Bai *et al.* (mean age 24 years old) who reported mean AXR in GM 4.71 ± 0.73 s^{-1} and WM 3.35 ± 0.78 s^{-1} in 7 healthy volunteers at the same b_f = 250s/mm^2.[224] Further BBB-FEXI imaging at a range of participant ages is required to understand whether BBB water exchange increases with age.

We were surprised to find as many as 50% of our cSVD cohort, none of whom had a prior cognitive diagnosis, had evidence of cognitive impairment on MoCA, requiring further evaluation. We showed interesting correlations between MoCA, BMET and our UoM online cognitive battery measurements of response speed and accuracy when compared to WMH volume. Given the prevalence of WMHs in our local population, there is a potentially unrecognised cohort with undiagnosed vascular cognitive impairment. We hope to validate our online battery further in a larger cohort of patients with cSVD including those with mild WMH burden as part of a future study. Given the significant findings relating to diffusivity

measurements, we have performed a post-hoc exploratory analysis for correlations between D_e and various cognitive parameters and include these in Supplementary Table 5-A.

We acknowledge that our study does have limitations. These are predominantly due to small participant numbers, partially due to the impact of COVID-19 during our study. Our CPs are significantly younger than the cSVD group and we have shown that age may impact upon WMH volume, D_e and K_b. This reflects the real-world recruitment difficulty of identifying individuals without significant WMHs in this age range, in whom, there are no other exclusion factors present. Given our small numbers, we are unable to adjust sufficiently for age difference but have included, where possible, performed paired analyses of the collective groups to minimise the impact. Participants were also grouped according to their conventional imaging, this was a pragmatic decision and replicated wider practice, but we acknowledge that the participants may have varied underlying pathologies. We present uncorrected *p* values, reflecting that our comparisons are not independent from one another and in keeping with the hypothesis generating, early stage of BBB-FEXI research.

Analysis of BBB-FEXI is based upon several assumptions, including blood volume for which we utilised fixed parameters in GM and WM. We acknowledge that blood volumes are inherently varied and may change between patients, regions (e.g., WM vs WMHs) and according to pathology. Combining BBB-FEXI with DCE-MRI in the future may ameliorate this concern as the blood volume can be directly measured. We also substituted literature values for T1/T2 where values were not available. We refer to Powell *et al.* for an estimation of bias introduced with these assumptions, but recognise that the assumed parameters can fixed across all subjects.[226] We constrained the recognised values for D_i, D_e and K_b, resulting in all voxels with extreme values being discarded. Where WMH volumes were low, this resulted in no

available values for 4 participants. These points add further weight to the requirement for standardisation in acquisition protocols, pre-processing and reporting of BBB-FEXI measurements.

In conclusion, there is a clear need to further our understanding of BBB imaging in cSVD, with the potential to support diagnosis, monitoring and the development of therapeutics. We have shown concerning trends between patient demographics, vascular risk scores and cognitive profiles with WMH volume and measures of microstructural integrity justifying further work in this area. Preclinical models may help to further the understanding of BBB dysfunction and the underlying mechanisms probed by each technique. Clinical studies are also required to make direct comparisons between imaging methodologies. Larger sufficiently powered studies are required to investigate the trends seen in K_b between WM and WMH alongside the effect of age and other clinical factors upon these novel parameters. If sensitive measures are identified, longitudinal studies should be performed to track the changes in cSVD natural history and assess their prognostic value in relation to cognitive decline, performance status and cardiovascular events.

Supplementary Table:

	GM D_e	WM D_e	WMH D_e
MoCA total	*r*= -0.65, *p*=0.0020**	*r*= -0.64, *p*=0.0024**	*r*=0.050, *p*=0.85
Abstraction	*r*= -0.0066, *p*=0.978	*r*= -0.15, *p*=0.54	*r*=0.14, *p*=0.62
Attention	*r*= -0.38, *p*=0.10	*r*= -0.36, *p*=0.12	*r*= -0.023, *p*=0.93
Delayed recall	*r*= -0.70, *p*=0.00064***	*r*= -0.65, *p*=0.0019**	*r*=0.10, *p*=0.71
Language	*r*= -0.60, *p*=0.0052**	*r*= -0.30, *p*=0.20	*r*=0.027, *p*=0.92
Naming	*r*= -0.13, *p*=0.58	*r*= -0.28, *p*=0.24	*r*= -0.08, *p*=0.76
Orientation	*r*= -0.44, *p*=0.054	*r*= -0.43, *p*=0.056	*r*= -0.08, *p*=0.76
Visuospatial / Executive	*r*= -0.49, *p*=0.030*	*r*= -0.63, *p*=0.0027**	*r*= -0.073, *p*=0.79
BMET total	*r*= -0.64, *p*=0.0025**	*r*= 0.59, *p*=0.0060**	*r*= -0.084, *p*=0.76
Memory	*r*= -0.062, *p*=0.0032**	*r*= -0.57, *p*=0.0081**	*r*= -0.094, *p*=0.73
Executive	*r*= -0.63, *p*=0.0027**	*r*= -0.59, *p*=0.0058**	*r*= -0.073, *p*=0.79
<u>UoM online cognitive battery:</u>			
Basic Recognition Task			
Response Time	*r*=0.64, *p*=0.0046**	*r*=0.45, *p*=0.006**	*r*=0.039, *p*=0.89
Accuracy	*r*= -0.18, *p*=0.47	*r*= -0.36, *p*=0.15	*r*= -0.35, *p*=0.22
Motor Task			
Response Time	*r*=0.56, *p*=0.016*	*r*=0.51, *p*=0.031*	*r*=0.28, *p*=0.32
Accuracy	n/a	n/a	n/a
Semantic Task			
Response Time	*r*=0.56, *p*=0.016*	*r*=0.40, *p*=0.10	*r*=0.072, *p*=0.81
Accuracy	*r*= -0.34, *p*=0.16	*r*= -0.36, *p*=0.15	*r*= -0.30, *p*=0.30
Visual Task			
Response Time	*r*=0.30, *p*=0.229	*r*=0.22, *p*=0.37	*r*= 0.27, *p*=0.35
Accuracy	*r*= -0.45, *p*=0.062	*r*= -0.39, *p*=0.11	*r*= -0.29, *p*=0.31

Supplementary Table 5-A: Pearson's correlation coefficient table for cognition and extravascular diffusivity (D_e) parameters. Abbreviations: GM: Grey Matter, WM: White Matter, WMH: White Matter Hyperintensity, MoCA: Montreal Cognitive Assessment, BMET: Brief Memory and Executive Task. Note: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Chapter 5 - Appendix List:

Appendix 5-1: BBB-FEXI pre-processing steps (courtesy of Dr Elizabeth Powell, UCL)

Chapter 6: Discussion and Conclusions

Contribution statement:
I am responsible for the design, drafting and amendments to this discussion.

Distribution:
Not applicable.

Cerebral small vessel disease (cSVD) is an impending public health emergency. Advances in medicine are contributing to an increasing lifespan, but are we also increasing the *healthy* life expectancy? As a society, we must wake up to the impact of cSVD, acknowledge the finite resources available to our National Health Service and seize the opportunity to change course.

From the outset of this thesis, I was aware of a perception of medical nihilism relating to cSVD. Using routinely collected data (RCD) we have shown just how prevalent these brain scan findings are. The headline figure is that WMHs are present upon almost 90% of brain scans performed in patients over the age of 80 years old here in Lancashire. We are not an outlier, with these figures aligning with large scale population studies.[40] It is clear that covert cSVD is a global problem in an ageing population.[5] However, we must not dismiss cSVD as being simply a disease of older adults, especially since we found almost 20% of patients under the age of 50 also have WMHs present. In the younger cohort, these WMHs were usually labelled as 'non-specific' in aetiology, though I suspect many were actually due to mild cSVD.

We know that cSVD may result in the potentially devastating clinical sequalae of stroke and dementia – this alone is surely enough to take action on a personal level and, of course,

preventative medicine can be economically advantageous on a population basis. In reality, though, the majority of cSVD is easily ignored.

It is commonly accepted that cSVD identified on brain imaging performed for other reasons is referred to as 'covert'. Firstly, I would like to make the case that incidental radiological cSVD is often not covert disease. In these patients, we may be overlooking subtle changes in cognition, mood and gait resulting in loss of independence, falls and institutionalisation.[11] From our RCD results we report that an increased World Health Organisation (WHO) performance score was associated with the presence of radiological cSVD – far from asymptomatic, these patients were more likely to be restricted in mobility and require assistance with their daily activities

This same finding was highlighted in our Water Exchange in the Vasculature of the Brain (WEX-BRAIN) results, alongside further associations with co-morbidity, smoking history and the number of medications prescribed. We had difficulty recruiting participants with moderate-severe cSVD due to *small vessel disease* occurring elsewhere, manifest with impaired renal function and preventing the safe administration of gadolinium-based contrast agents. Of the 10 participants with cSVD, none of whom had a prior cognitive diagnosis, 50% achieved a score on the Montreal Cognitive Assessment (MoCA) compatible with cognitive impairment. WMH volume correlated with reduced performance on the MoCA, Brief Memory and Executive Task (BMET) and with our online assessment of processing speed and executive functioning.

We also noted that increased QRISK3, a composite vascular risk score used to measure the probability of having a myocardial infarction or stroke in the next 10 years, was associated with increasing WMH volume. To my knowledge, this has not previously been reported and

serves to highlight the importance of *incidental* WMHs as a marker of overall cardiovascular risk. Further work is required in this area, as we note that advancing age has a significant impact on both the prevalence of radiological WMHs and QRISK3 score. I am hoping to apply the skills I have developed during this PhD to further investigate this association using routinely collected data.

Despite all of the above, I found recruiting patients with cSVD to the WEX-BRAIN study unexpectedly (*perhaps naively*) difficult. The COVID-19 pandemic may have been contributory, but healthy volunteers were comparatively eager to undergo an MRI brain. I was frequently met with resistance from potential cSVD participants, realising quickly that patients were often unaware of the WMHs on their brain scan. Far from a call to action, cSVD changes had often been dismissed, minimised or miscommunicated. To the contrary, some patients had been erroneously informed they had suffered a 'stroke' and had been established on unnecessary or potentially harmful preventative medications.

I realised that there was a clear unmet need relating to patient education in this domain, which has changed my practice as a neurologist. I now insert standardised text into results letters to patients, explaining WMHs in more detail – focussing on the simple interventions recommended by the ESO covert cSVD guideline such as advising patients to have their blood pressure checked, to follow a healthy lifestyle and, where appropriate, advising upon smoking cessation. I hope to more formally address this in the future as a local quality improvement programme, understanding the breadth of local practice and then – with input from patients and the general public – produce a range of educational materials such as leaflets, online information or an educational podcast/video.

Joining Dr Sen in his specialist cSVD clinic at Salford Royal Hospital (Northern Care Alliance) was a landmark moment for WEX-BRAIN recruitment, encountering well-informed and often highly motivated patients both ready to enact the recommended lifestyle changes and participate in research studies. I was also fortunate to become accustomed to communicating the findings of cSVD imaging (including sharing the scan images with patients), assessing for the various clinical syndromes associated with cSVD, categorising the suspected aetiology and performing any appropriate further investigations (e.g., genetic testing). I recognised that patients with more severe cSVD often appeared 'frail', and I do suspect many of the studies which have used frailty as a measure in *asymptomatic* cSVD were probably measuring a degree of *symptomatic* cSVD from the outset.[353]

The next challenge for this relatively novel cSVD clinic will be judging the efficacy of these interventions upon patient outcomes. Given the natural history of cSVD this will take several years and a large cohort of patients. I hope to firstly complete a service evaluation to capture the routinely collected data from this clinic and understand the cohort in more detail, audit the performance against the ESO guideline and, if possible, apply some of my recently acquired semi-automated quantitative imaging analysis skills to their relevant follow up imaging to judge the impact upon WMH progression. The recent study demonstrating that WMHs can progress as well as regress further supports this endeavour.[44] In the meantime, the work contained within this thesis has supported a recent business case to set up a local cSVD clinic here in Lancashire. We hope this will also serve as a springboard for several local research studies for patient benefit including collaboration with our imaging and data science colleagues in academia and industry.

Interest in the BBB has never been greater. This includes the BBB as a diagnostic tool, a therapeutic target and, from a different perspective, the BBB as an obstacle. Indeed, there is now a wealth of literature relating to the deliberate induction of targeted BBB disruption using techniques such as focussed ultrasound to facilitate drug delivery into CNS.[354] I have no doubt that further research into BBB structure, function and its role in various pathologies is justified.

However, several unanswered questions remain regarding BBB water exchange and utilising this as a clinical marker of BBB dysfunction. The precise mechanism by which water crosses the BBB in health and disease is not yet established. We have yet to understand the complete role of aquaporin 4, located beyond the basement membrane of brain endothelial cells, despite it appearing a crucial mediator of water homeostasis in animal and human models. Furthermore, water exchange in the central nervous system (CNS) does not occur solely at the BBB, taking place also at the blood-cerebrospinal fluid (CSF) barrier at the choroid plexus and in constant flux with the glymphatic system via perivascular spaces. At present, these processes are often considered in isolation, despite BBB water exchange contributing only a relatively small fraction of overall water flux into the CNS.

When we consider the application to individual patients, *small scale* studies become n=1 and our systematic scoping review revealed significant heterogeneity in image acquisition, processing and reporting of human studies as expected in the early stages of a novel technology. I am acutely aware of the healthy scepticism relating to many methodologies.[168,210] I remain unconvinced regarding the clinical application of whole brain water exchange measurements, given the lack of topographical information to identify the site of BBB dysfunction and the potential for water exchange to increase or decrease regionally – limiting the findings of

averaged values. To our knowledge, there is currently no validated method able to perform accurately on a whole brain, voxel-wise basis.

Nevertheless, the studies included in our review did demonstrate some accurate and repeatable results, with BBB water exchange studies now reported in a variety of pathologies. There is certainly impetus to continue to fully understand and validate these processes in larger cohorts and longitudinal studies. I agree with authors who suggest that performing several methods in tandem is required, at least initially, to reduce the number of assumed values (e.g., blood volume) and to assist with interpretation of findings. I would highlight that Gadolinium based contrast agents are becoming less of a concern with newer preparations.

The results of WEX-BRAIN may, at first glance, appear underwhelming. There was no significant difference in BBB water exchange (K_b) between the grey matter, white matter or WMHs in participants with cSVD when compared with healthy participants. There are several potential reasons behind this – namely though the study is likely to be underpowered (given the differences in BBB water exchange being measured and the number of confounding variables in an older population) and the wider WEX-BRAIN team continue to optimise the post-processing of the BBB-FEXI imaging. Nevertheless, WEX-BRAIN was always an exploratory, hypothesis generating study and to our knowledge is the first study of its kind to perform BBB-FEXI applying the latest recommendations for 2-compartment modelling and accounting for T1/T2 tissue relaxivity in cSVD. This was also achieved using only clinically available hardware.

We also identified interesting results with respect to the extravascular (tissue) diffusivity measurements (D_e), showing a difference in D_e between the normal appearing WM of cSVD

participants and the WM of healthy controls (0.771 ± 0.0624 vs. 0.661 ± 0.0530 um^2ms, respectively $p<0.01$). This correlated well with measurements based upon Diffusion Tensor Imaging and we hypothesise this represents impaired microstructural integrity of what appears on conventional imaging as normal white matter in patients with cSVD. Whist there has been some reporting of these parameters previously (referred to as ADC_0),[281] I am not aware of any extended-interval longitudinal studies and it would be fascinating to know whether this could predict the subsequent appearance of WMHs on conventional imaging.

Two significant changes were made to the WEX-BRAIN protocol, which do contradict the recommendations made throughout this thesis. The first relates to performing multiple assessments of BBB dysfunction for comparative purposes. We had intended to perform DCE-MRI alongside the BBB-FEXI analysis. However, challenges with recruitment in the context of COVID-19, a cyberattack, scanner availability and renal function thresholds prevented this. Personal communication with Dr S Al-Bachari reveals that our local research team has performed DCE-MRI in a cohort of patients with cSVD and found no significant difference when compared to healthy controls (as part of a larger study focussed on Parkinson's disease). Nevertheless, capturing the DCE-MRI data would have provided a measurement of blood volume, which we have assumed according to literature values for white matter and grey matter – which is unlikely to be uniform across all participants and throughout the brain. Secondly, we recognise the value of longitudinal imaging. We had hoped to perform a follow up visit to participants after 12-18 months to assess for clinical sequalae of cSVD and, if additional funding was available, repeat their brain scan for comparison. Due to time constraints, this was not possible.

Being EPSRC funded, the WEX-BRAIN study was eligible for NIHR Portfolio adoption. This presented the opportunity to work closely with the research teams at Lancashire NIHR Clinical Research Facility. Alongside a valuable source of wisdom, support and expertise in conducting the WEX-BRAIN study, I was able to obtain valuable insight into the challenges facing studies – for example, with study recruitment – and make the necessary amendments efficiently. I completed the NIHR Associate Principal Investigator scheme during this period whilst working on the Lighthouse II study, increasing the availability of research opportunities for patients here in Lancashire.

In conclusion, I am steadfast in my opinion that cSVD is very common and very important. Whilst we do not have specific disease modifying therapies available, our clinical priority should be to communicate the findings of WMHs and cSVD to patients more effectively and develop services to screen for modifiable vascular risk factors and to both diagnose and optimise the management of hypertension. In the meantime, further research is required to understand the role of BBB dysfunction in cSVD and the physiological and pathological parameters of water exchange. Given the potential benefits in terms of diagnostics, monitoring and supporting the development of therapeutics, the findings from this work would suggest that larger longitudinal studies are needed to determine the sensitivity of measurements to track cSVD pathology and test their prognostic value for clinical parameters such as cognitive decline.

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EBSCOhost

Wednesday, October 20, 2021 1:41:05 PM

#	Query	Limiters/Expanders	Last Run Via	Results
S4	s1 and s2 and s3	Search modes - Find all my search terms	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - MEDLINE Complete	Display
S3	((MH "Blood-Brain Barrier") OR (MH "Blood Vessels+")) OR TI ((("blood-brain" OR "blood brain" OR "bloodbrain" OR "brain-blood" OR "brain blood" OR "brainblood") N3 Barrier) OR (("vessel*" OR "vascula*" OR "arter*" OR "capillar*" OR "microvascula*") N3 ("brain" OR "intracerebral" OR "cerebral")) OR AB ((("blood-brain" OR "blood brain" OR "bloodbrain" OR "brain-blood" OR "brain blood" OR "brainblood") N3 Barrier) OR (("vessel*" OR "vascula*" OR "arter*" OR "capillar*" OR "microvascula*") N3 ("brain" OR "intracerebral" OR "cerebral"))	Search modes - Find all my search terms	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - MEDLINE Complete	Display
S2	TI (("permeability" OR "diffusi*" OR "exchange*" OR "transfer*" OR "extraction" OR "efflux" OR "leakage") N3 ("water" OR "H2O")) OR AB (("permeability" OR "diffusi*" OR "exchange*" OR "transfer*" OR "extraction" OR "efflux" OR "leakage") N3 ("water" OR "H2O"))	Search modes - Find all my search terms	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - MEDLINE Complete	Display
S1	((MH "Magnetic Resonance Imaging+") OR (MH "Diffusion Magnetic Resonance Imaging+") OR (MH "Multiparametric Magnetic Resonance Imaging") OR (MH "Magnetic Resonance Angiography") OR (MH "Diffusion Tensor Imaging")) OR TI (MRI OR "MR" OR M.R.I* OR magnetic resonance OR magnetic resonance imag*) OR AB (MRI OR "MR" OR M.R.I* OR magnetic resonance OR magnetic resonance imag*)	Search modes - Find all my search terms	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - MEDLINE Complete	Display

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Lancashire Teaching Hospitals
NHS Foundation Trust

PARTICIPANT INFORMATION SHEET

You are being invited to participate in a research study led by Professor H. Emsley and his team at Lancashire Teaching Hospitals NHS Foundation Trust. Please read the following information carefully before you decide whether to take part in the study or not.

WHAT IS THE PURPOSE OF THIS STUDY?

The brain needs a constant supply of energy and oxygen to work effectively. These are delivered by the bloodstream, which also removes waste products. The blood-brain barrier separates the blood vessels from brain tissue and allows essential molecules to rapidly cross the barrier, but harmful chemicals cannot enter the brain. In patients with a stroke, the blood-brain barrier is broken and this causes damage to the brain. An important cause of stroke is a condition called small vessel disease (disease affecting the tiny vessels which supply brain tissue) and this can also lead to problems with memory.

Currently, we have no suitable method of assessing blood-brain barrier function in living patients. Being able to measure its function would allow us to better diagnose, monitor and potentially develop new treatments for a range of problems affecting the brain.

Our research is developing new ways of assessing blood-brain barrier function using MRI scans, focusing on how water is able to pass across this barrier. This is called the 'water exchange' method and we will be comparing this to existing techniques which use Gadolinium contrast agents (dyes which enhance features of the scan).

We have already begun testing these new types of scans in the lab. We intend to trial these new measurements for the first time in a range of participants, including those who have recently had a stroke and in those with small vessel disease.

Alongside the MRI scanning, we will also be performing memory tests so that we can better understand the clinical impact of blood-brain barrier function.

WHY HAVE I BEEN CHOSEN?

In total, we aim to recruit 80 participants. You have been chosen either because you have recently had a stroke, or because you have evidence of cerebral small vessel disease on your previous MRI brain scan.

Our scans will require the use of a contrast agent, therefore we will need to ensure that you do not have an allergy to 'gadolinium-based' contrast agents and we will need to check your kidney function prior to the scan with a blood test.

As part of the recruitment process, we will need to ask you if you have any history of recreational drug use as we know this can impact on the function of the blood-brain barrier.

WHAT WILL HAPPEN IF I TAKE PART?

If you decide to take part in the study, we will ask you to participate in 3 visits.

Visit 1 will usually take place as a home visit, scheduled at a time which is convenient for you. We will complete a questionnaire regarding your medical history and perform some memory tests. We will also take a blood test to measure your kidney function. The latest social distancing guidance will be followed during these visits. In certain circumstances, we can offer alternatives to a home visit including attending the research facility at Royal Preston Hospital / Salford Royal Hospital or completing a limited assessment during a telephone call.

Visit 2 will involve having a magnetic resonance imaging (MRI) scan of your brain and will be completed at either Salford Royal Hospital or at the Manchester Wellcome Trust/NIHR Clinical Research Facility on Grafton Street in Manchester. If your initial assessment was by telephone, we may need to take a blood sample on the day of your scan or arrange to collect this a few days beforehand.

On arrival, we will check that it is safe for you to enter the scanner (you will need to remove all metallic items such as belts, phones and keys) and we will ask a few additional questions such as whether you are left or right-handed. We will insert a cannula to allow us to give a contrast injection during the scan. The MRI scan itself is painless, safe and will take approximately 1 hour to complete. In total, we expect this visit to take 2 hours (excluding travel).

Visit 3 will be arranged approximately 12-18 months after your initial assessment. We will contact you by telephone to arrange a convenient time. During this visit we will repeat your memory tests and confirm any changes in your medical history. Depending on the initial results of our study, we are planning to invite some patients to have a further MRI scan at this stage, which would follow a very similar process to your previous scan. This additional scan would be completely optional but would likely require a further blood test before proceeding.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

There are no direct benefits for you through taking part in this study. However, we hope that the information that we gain will help to improve our understanding of the blood-brain barrier and the relationship with small vessel disease, stroke and memory.

We will pay for your transport to and from the scan or arrange a taxi for you if this is preferable. We can also offer a small payment of £10 to compensate for your time at each visit.

WHAT ARE THE POSSIBLE RISKS OF TAKING PART?

MRI scans are a safe and painless procedure. MRI uses a strong magnet, so we will make sure that it is safe for you to go into the scanner (e.g. you don't have any metal implants or a pacemaker). Before going into the scanner, you will need to remove any items containing metal such as cash, a belt, jewellery or a watch. You may be asked to change into a gown, if necessary.

For one of the scans, a dye (contrast agent) is required. There is very low risk of reaction to the contrast agent. Major allergic reactions are rare, occurring in one in one thousand cases or fewer. Inserting a cannula into a vein carries a slim risk of infection and bruising, but this is rare.

Scans will be carried out by an experienced radiographer. The scanner experience is noisy, and has been reported as 'claustrophobic', causing anxiety in some individuals. If you experience any distress, the scanning process will be immediately discontinued.

Incidental findings (unexpected findings which are often unimportant) are sometimes identified on MRI scans. In the event of incidental findings or if we have any concerns regarding the results, we will notify your GP with advice on further management, if appropriate.

HOW WILL MY PERSONAL INFORMATION BE USED?

Lancashire Teaching Hospitals NHS Foundation Trust is the Sponsor for this study. Recruitment is also taking place at Salford Royal NHS Foundation Trust. You will have been identified as suitable for inclusion within the study by your doctor.

We will need to use information from you and your medical records for this research project. This information will include your initials, NHS number, name, contact details, medical history, kidney function and memory tests.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a study identification number instead.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

WILL MY TAKING PART BE KEPT CONFIDENTIAL?

Yes, taking part in the study will be confidential. We will assign you an individual identification code (or Study ID) so that researchers analysing your information will not be able to identify you and will not have access to your name and contact information.

We will only access personal information such as your name and contact details when we need to make contact with you at the pre-arranged time points. Individuals from Lancashire Teaching Hospitals/Salford Royal NHS Foundation Trust and regulatory authorities may need to review your research records to check the accuracy of this study.

Your GP will be informed regarding your involvement in the study and will be contacted if your MRI brain scan shows evidence of important incidental findings or if you are identified to have major depression.

WHAT ARE YOUR CHOICES ABOUT HOW YOUR INFORMATION IS USED?

You can stop being part of the study at any time, without giving a reason, but we will keep anonymised information about you that we already have indefinitely.

We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

You can find out more about how we use your information at:
https://www.hra.nhs.uk/information-about-patients/ or by contacting the researcher.

WHO SHOULD I CONTACT IF I AM UNHAPPY WITH MY TREATMENT AND WISH TO MAKE A COMPLAINT.

If you have a specific concern or query about the research then you can contact the study team using the details below.

For a more independent contact, you can contact the Research Governance lead within the Centre for Health Research and Innovation at Lancashire Teaching Hospitals by contacting 01772 522031.

You may also wish talk to the hospital Patient Advice and Liaison Service (PALS) which provides support to patients, families and visitors. Hopefully, in most cases they will be able to sort out your concerns very quickly. However, if you are not satisfied with the response that you receive you can make a complaint in writing. Please contact the Trust's Customer Care department on 01772 522521 or email customer.care@lthtr.nhs.uk who can assist you with your complaint.

Contact details for further information

Dr Mark Maskery (PhD student)
Department of Neurology, Royal Preston Hospital, Sharoe Green Lane, Preston, PR2 9HT
Mark.Maskery@LTHTr.nhs.uk

Lancashire Teaching Hospitals
NHS Foundation Trust

Royal Preston Hospital,
Sharoe Green Lane,
Fulwood,
Preston.
PR2 9HT

Patient Identification Number for this trial:

CONSENT FORM

Title of Project: WEX-BRAIN – Water EXchange in the vasculature of the BRAIN

Name of Researcher: Dr Mark Maskery

Please initial box

1. I confirm that I have read and understand the participant information sheet (v1.2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. I understand that it will not be possible to remove my data from the project once it has been anonymised and forms part of the data set. I agree to take part on this basis ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the study team, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in the study and any incidental abnormality identified on my scans will be reported to my medical general practitioner (GP). I agree to my GP being informed if I am found to have major depression when screened. ☐

5. I understand that data collected from me for this study will be used by employees of Lancashire Teaching Hospitals, Lancaster University and The University of Manchester for the purpose of research, and potentially by employees of regulatory authorities for oversight purposes. ☐

6. I understand that the sponsors of this study may make my brain images available to other researchers for future research and that this may include researchers working abroad. I give permission for these individuals to have access to my brain images, but not any personal identifying information about me. ☐

7. I agree that any data collected may be published in anonymous form in academic books, reports, journals or abstracts. ☐

8. I agree for my anonymous data to be retained indefinitely for further brain imaging research ☐

9. I understand the risks associated with and agree to intravenous injection of gadolinium-based contrast agents ☐

10. I agree to take part in this study. ☐

11. I agree to be contacted for future imaging studies *(optional)* ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

Amendment Tool

For office use

QC: No

Section 1: Project information

Short project title*:	Blood brain barrier dysfunction in cerebral small vessel disease
IRAS project ID* (or REC reference if no IRAS project ID is available):	297513
Sponsor amendment reference number*:	
Sponsor amendment date* (enter as DD/MM/YY):	
Briefly summarise in lay language the main changes proposed in this amendment. Explain the purpose of the changes and their significance for the study. If the amendment significantly alters the research design or methodology, or could otherwise affect the scientific value of the study, supporting scientific information should be given (or enclosed separately). Indicate whether or not additional scientific critique has been obtained (note: this field will adapt to the amount of text entered)*:	1) Currently, participants/healthy volunteers undergo a blood test to measure kidney function (eGFR) prior to having an MRI brain with contrast, this blood test must be completed within 3 months of the scan with eGFR >60 in order to proceed. We propose that, for healthy volunteers or participants with a prior normal kidney function test instead have the option to undergo a bedside eGFR measurement on the day of scanning instead of a formal blood test in advance. If the bedside eGFR is <60 then we will delay the scan and the participant will undergo a formal blood test prior to considering a scan. 2) Currently participants are recruited via a single centre, we propose to also recruit participants from an additional 'participant identification centre', namely Salford Royal NHS Foundation Trust. 3) Addition of local PI (Dr Sen) at Salford Royal NHS Foundation Trust 4) Current approvals include repeat cognitive (memory) assessment at 12-18 months following initial recruitment, we propose to invite selected participants (<20) to return for a further MRI scan. 5) In view of social distancing measures, we propose that participants will have the option to adjust visit 1 from a home visit to a telephone visit - therefore demongraphics/medical questionnaire will be completed by telephone consultation, with cognitive screening reduced to only MoCA. 6) We had previously intended to recruit a total of 60 participants (20 healthy volunteers, 20 small vessel disease and 20 with small vessel disease and a recent stroke) based upon ongoing discussion with participants, the results of our systematic scoping review, analysis of initial results and the challenges posed by COVID-19 on recruitment we propose to continue to recruit 60 participants, but with flexibility in the small vessel disease (n=40) category.

Project type (select):	**Specific study**			
	Research tissue bank			
	Research database			
Has the study been reviewed by a UKECA-recognised Research Ethics Committee (REC) prior to this amendment?:	**Yes**		No	
What type of UKECA-recognised Research Ethics Committee (REC) review is applicable? (select):	**NHS/HSC REC**			
	Ministry of Defence (MoDREC)			
Is all or part of this amendment being resubmitted to the Research Ethics Committee (REC) as a **modified amendment** (i.e. a substantial amendment previously given an unfavourable opinion)?	Yes		**No**	
Where is the NHS/HSC Research Ethics Committee (REC) that reviewed the study based?:	England	Wales	Scotland	Northern Ireland
	Yes	No	No	No
Was the study a clinical trial of an investigational medicinal product (CTIMP) OR does the amendment make it one?:	Yes		**No**	
Was the study a clinical investigation or other study of a medical device OR does the amendment make it one?:	Yes		**No**	
Did the study involve the administration of radioactive substances, therefore requiring ARSAC review, OR does the amendment introduce this?:	Yes		**No**	
Did the study involve the use of research exposures to ionising radiation (not involving the administration of radioactive substances) OR does the amendment introduce this?:	Yes		**No**	
Did the study involve adults lacking capacity OR does the amendment introduce this?:	Yes		**No**	
Did the study involve access to confidential patient information outside the direct care team without consent OR does the amendment introduce this?:	Yes		**No**	
Did the study involve prisoners or young offenders who are in custody or supervised by the probation service OR does the amendment introduce this?:	Yes		**No**	
Did the study involve children OR does the amendment introduce this?:	Yes		**No**	
Did the study involve NHS/HSC organisations prior to this amendment?:	**Yes**		No	
Did the study involve non-NHS/HSC organisations OR does the amendment introduce them?:	Yes		**No**	
	England	Wales	Scotland	Northern Ireland
Lead nation for the study:	**Yes**	No	No	No
Which nations had participating NHS/HSC organisations prior to this amendment?	**Yes**	No	No	No
Which nations will have participating NHS/HSC organisations after this amendment?	**Yes**	No	No	No
Was this a "single site, self sponsored" study in England or Wales prior to this amendment?	Yes		**No**	

Section 2: Summary of change(s)

Please note: Each change being made as part of the amendment must be entered separately. For example, if an amendment to a clinical trial of an investigational medicinal product (CTIMP) involves an update to the Investigator's Brochure (IB), affecting the Reference Safety Information (RSI) and so the information documents to be given to participants, these should be entered into the Amendment Tool as three separate changes. A list of all possible changes is available on the "Glossary of Amendment Options" tab. To add another change, click the "Add another change" box.

Change 1

Area of change (select)*:	Participant Procedures
Specific change (select - only available when area of change is selected first)*:	Participant procedures - minor change that will have additional resource implications for participating organisations - Please specify in the free text below
Further information In particular, please describe the additional resource arrangements that participating organisations will need to have in place to implement this change (free text - note that this field will adapt to the amount of text entered)*:	Currently, participants undergoing an MRI brain with contrast as part of the study protocol require a blood test for eGFR (kidney function), with a result of >60 (adjusted for Afro-carribean populations) within 3 months of the scanning date. For healthy volunteers (where we would not expect kidney problems) or participants who have had a recent (but >3 months) eGFR >60 we will have the option to instead perform a bedside eGFR measurement on the day of scanning. If this bedside result is >60 then we will proceed with the scan. If the result is <60 then the scan will be delayed and a standard kidney function blood test will be completed. All scans are currently being performed as a single scanning location, Salford Royal Hospital. A bedside eGFR device is already being used for other local studies and the equipment will be available for use by our study if this amendment is approved. We acknowledge that the vast majority of participants will continue to have a blood test completed in advance, in line with current approvals, but this amendment will help to mitigate the delays we have encountered in light of COVID-19 pressures.

Applicability:	England	Wales	Scotland	Northern Ireland
Where are the participating NHS/HSC organisations located that will be affected by this change?*:	**Yes**	No	No	No
Will all participating NHS/HSC organisations be affected by this change, or only some? (**please note** that this answer may affect the categorisation for the change):	**All**		Some	
			Remove all changes below	

Change 2	
Area of change (select)*:	Participating Organisations
Specific change (select - only available when area of change is selected first)*:	Addition of sites undertaking the same activities as existing sites
Further information (free text - note that this field will adapt to the amount of text entered):	Currently, participants are recruited from Royal Preston Hospital (Lancashire Teaching Hospitals NHS Foundation Trust). We intend to add a further 'participant identification centre' at Salford Royal NHS Foundation Trust. Due to the impact of the COVID-19 pandemic, which could not be anticipated at the time of study conception, we acknowledge (through discussions with our study population) a change in behaviour relating to willingness to travel to Salford for the scan. By recruiting locally in Salford we aim to improve the recruitment to our study whilst not impacting on the overall scientific rigour of results.

Applicability:	England	Wales	Scotland	Northern Ireland
Where are the participating NHS/HSC organisations located that will be affected by this change?*:	**Yes**	No	No	No
Will all participating NHS/HSC organisations be affected by this change, or only some? (**please note** that this answer may affect the categorisation for the change):	**All**		Some	
			Remove all changes below	

Change 3	
Area of change (select)*:	Researchers
Specific change (select - only available when area of change is selected first)*:	PI - New PI, or temporary arrangements to cover the absence of a PI
Further information (free text - note that this field will adapt to the amount of text entered):	Include Dr Dwaipayan Sen, consultant stroke physician as local PI for the additional site at Salford Royal NHS Foundation Trust

Applicability:	England	Wales	Scotland	Northern Ireland
Where are the participating NHS/HSC organisations located that will be affected by this change?*:	**Yes**	No	No	No
Will all participating NHS/HSC organisations be affected by this change, or only some? (**please note** that this answer may affect the categorisation for the change):	All		**Some**	
			Remove all changes below	

Change 4	
Area of change (select)*:	Participant Procedures
Specific change (select - only available when area of change is selected first)*:	Participant procedures - significant change that can be implemented within existing resource at participating organisations - Please specify in the free text below
Further information In particular, please describe why this change can be implemented within the existing resource in place at the participating organisations (free text - note that this field will adapt to the amount of text entered)*	Participants currently undergo repeat cognitive testing at 12-18 month interval following their MRI brain scan to assess for evidence of cognitive decline. We propose that certain participants (determined by initial imaging results, interim medical history or cognitive performance) will be invited for a 2nd MRI brain scan. These participants will undergo repeat kidney function testing (with eGFR required to be >60) if they are to receive gadolinum based contrast agents. This amendment will maximise the output from participant enrollment, providing longitudinal small vessel disease/cognitive data not currently available in the scientific literature and strengthening overall scientific impact of study (see our systematic scoping review).

Applicability:	England	Wales	Scotland	Northern Ireland
Where are the participating NHS/HSC organisations located that will be affected by this change?*:	**Yes**	No	No	No
Will all participating NHS/HSC organisations be affected by this change, or only some? (**please note** that this answer may affect the categorisation for the change):	**All**		Some	

Remove all changes below

Change 5	
Area of change (select)*:	Participant Procedures
Specific change (select - only available when area of change is selected first)*:	Participant procedures - minor change that can be implemented within existing resource at participating organisations - Please specify in the free text below
Further information In particular, please describe why this change can be implemented within the existing resource in place at the participating organisations (free text - note that this field will adapt to the amount of text entered)*	Currently, visit 1 is carried out as a home visit. A local researcher visits the participants home to complete the consent form, medical history, cognitive testing and blood test. We are finding that some participants are reluctant to accept home visits in view of the recent social distancing requirements and would prefer to avoid attending the CRF at Royal Preston Hospital due to the perceived risk of exposure to COVID-19. We envisage this to improve over time, however, it is currently having a negative impact on our ability to recruit participants. We therefore wish to offer an option of conducting visit 1 as a telephone consultation, at a convenient time for the participant. Therefore, the cognitive testing would no longer be completed, except for a telephone Montreal Cognitive Assessment (MoCA). The participant would either have kidney function bloods already available in the last 3 months (with eGFR >60) or, if they have prior normal results (>3 months ago) they would have a bedside eGFR measurement on the day of the scan (see change 1, above). If neither option is suitable, a short visit will be arranged for blood tests only. The consent form would be completed by telephone at visit 1 and signed at visit 2 (scanning visit).

Applicability:	England	Wales	Scotland	Northern Ireland
Where are the participating NHS/HSC organisations located that will be affected by this change?*:	**Yes**	No	No	No
Will all participating NHS/HSC organisations be affected by this change, or only some? (**please note** that this answer may affect the categorisation for the change):	**All**		Some	

Remove all changes below

Change 6	
Area of change (select)*:	Study Design
Specific change (select - only available when area of change is selected first)*:	Other minor change to study design that can be implemented within existing resource in place at participating organisations - Please specify in the free text below
Further information In particular, please describe why this change can be implemented within the existing resource in place at the participating organisations (free text - note that this field will adapt to the amount of text entered)*	We recognise, through experience of early recruitment and analysis of preliminary and optimisation scans that the requirement for precisely matched groups of small vessel disease and stroke is not crucial for the scientific value of the study. Indeed, after discussion with relevant experts in the field we are likely to find a range of small vessel disease (hypertensive, CAA, lacunar infarcts) and severity of stroke more beneficial for the value of this study. Currently we propose to recruit 20 participants with moderate to severe small vessel disease and 20 participants with moderate to severe small vessel disease and a recent large artery infarct (stroke). We wish to amend this to include 40 participants with a range of moderate to severe small vessel disease and large artery stroke. We recognise due to the heterogeneity of small vessel disease encountered during routine clinical practice that this may prospectively or retrospectively include both sporadic and genetic small vessel disease participants, but acknowledge that this is in line with the exploratory nature of the current study and exisiting literature.

Applicability:	England	Wales	Scotland	Northern Ireland
Where are the participating NHS/HSC organisations located that will be affected by this change?*:	**Yes**	No	No	No
Will all participating NHS/HSC organisations be affected by this change, or only some? (**please note** that this answer may affect the categorisation for the change):	**All**		Some	

Add another change

Section 3: Declaration(s) and lock for submission

Declaration by the Sponsor or authorised delegate

- I confirm that the Sponsor takes responsibility for the completed amendment tool
- I confirm that I have been formally authorised by the Sponsor to complete the amendment tool on their behalf

Name [first name and surname]:*	
Email address:*	

Lock for submission

Please note: This button will only become available when all mandatory (*) fields have been completed. When the button is available, clicking it will generate a locked PDF copy of the completed amendment tool which must be included in the amendment submission. Please ensure that the amendment tool is completed correctly before locking it for submission.

Lock for submission

After locking the tool, proceed to submit the amendment online. The "Submission Guidance" tab provides further information about the next steps for the amendment.

Section 4: Review bodies for the amendment

Please note: This section is for **information only**. Details in this section will complete automatically based on the options selected in Sections 1 and 2.

Review bodies

	UK wide:						England and Wales:				Scotland:				Northern Ireland:				
	REC	Competent Authority MHRA - Medicines	Competent Authority MHRA - Devices	ARSAC	Radiation Assurance	UKSW Governance	REC (MCA)	CAG	HMPPS	HRA and HCRW Approval	REC (AWIA)	PBPP	SPS (RAEC)	National coordinating function	HSC REC	HSC Data Guardians	Prisons	National coordinating function	Category:
Change 1:	N					(Y)				(Y)									A
Change 2:	N					(Y)				(Y)									New site
Change 3:	N					(Y)				(Y)									B
Change 4:	Y					Y				Y									C
Change 5:	N					(Y)				(Y)									C
Change 6:	N					(Y)				(Y)									C
Overall reviews for the amendment:																			
Full review:	Y					Y				Y									
Notification only:	N					N				N									
Overall amendment type:	Substantial																		
Overall Category:	A																		

Amendment Tool

v1.6 06 December 2021

For office use

QC: No

Section 1: Project information

Short project title*:	Blood brain barrier dysfunction in cerebral small vessel disease
IRAS project ID* (or REC reference if no IRAS project ID is available):	297513
Sponsor amendment reference number*:	NSA01
Sponsor amendment date* (enter as DD/MM/YY):	24 January 2023
Briefly summarise in lay language the main changes proposed in this amendment. Explain the purpose of the changes and their significance for the study. If the amendment significantly alters the research design or methodology, or could otherwise affect the scientific value of the study, supporting scientific information should be given (or enclosed separately). Indicate whether or not additional scientific critique has been obtained (note: this field will adapt to the amount of text entered)*:	To reflect what is now becoming widespread research and clinical practice elsewhere, and in view of the current available evidence, we propose to lower the kidney function threshold for contrast administration in MRI scanning (from eGFR >60 to eGFR >40) to include additional participants with significant cerebral small vessel disease.

Project type (select):	**Specific study**			
	Research tissue bank			
	Research database			
Has the study been reviewed by a UKECA-recognised Research Ethics Committee (REC) prior to this amendment?:	**Yes**		No	
What type of UKECA-recognised Research Ethics Committee (REC) review is applicable? (select):	**NHS/HSC REC**			
	Ministry of Defence (MoDREC)			
Is all or part of this amendment being resubmitted to the Research Ethics Committee (REC) as a **modified amendment** (i.e. a substantial amendment previously given an unfavourable opinion)?	Yes		**No**	
Where is the NHS/HSC Research Ethics Committee (REC) that reviewed the study based?:	England	Wales	Scotland	Northern Ireland
	Yes	No	No	No
Was the study a clinical trial of an investigational medicinal product (CTIMP) OR does the amendment make it one?:	Yes		**No**	
Was the study a clinical investigation or other study of a medical device OR does the amendment make it one?:	Yes		**No**	
Did the study involve the administration of radioactive substances, therefore requiring ARSAC review, OR does the amendment introduce this?:	Yes		**No**	
Did the study involve the use of research exposures to ionising radiation (not involving the administration of radioactive substances) OR does the amendment introduce this?:	Yes		**No**	
Did the study involve adults lacking capacity OR does the amendment introduce this?:	Yes		**No**	
Did the study involve access to confidential patient information outside the direct care team without consent OR does the amendment introduce this?:	Yes		**No**	
Did the study involve prisoners or young offenders who are in custody or supervised by the probation service OR does the amendment introduce this?:	Yes		**No**	
Did the study involve children OR does the amendment introduce this?:	Yes		**No**	
Did the study involve NHS/HSC organisations prior to this amendment?:	**Yes**		No	
Did the study involve non-NHS/HSC organisations OR does the amendment introduce them?:	Yes		**No**	
	England	Wales	Scotland	Northern Ireland
Lead nation for the study:	**Yes**	No	No	No
Which nations had participating NHS/HSC organisations prior to this amendment?	**Yes**	No	No	No
Which nations will have participating NHS/HSC organisations after this amendment?	**Yes**	No	No	No
Was this a "single site, self sponsored" study in England or Wales prior to this amendment?	Yes		**No**	

Section 2: Summary of change(s)

Please note: Each change being made as part of the amendment must be entered separately. For example, if an amendment to a clinical trial of an investigational medicinal product (CTIMP) involves an update to the Investigator's Brochure (IB), affecting the Reference Safety Information (RSI) and so the information documents to be given to participants, these should be entered into the Amendment Tool as three separate changes. A list of all possible changes is available on the "Glossary of Amendment Options" tab. To add another change, click the "Add another change" box.

Change 1	
Area of change (select)*:	Participant Procedures
Specific change (select - only available when area of change is selected first)*:	Participant procedures - minor change that can be implemented within existing resource at participating organisations - Please specify in the free text below
Further information In particular, please describe why this change can be implemented within the existing resource in place at the participating organisations (free text - note that this field will adapt to the amount of text entered)*	The WEX-BRAIN study is evaluating novel MRI based methods of measuring blood-brain barrier dysfunction in patients with cerebral small vessel disease, stroke and a control population. As part of the imaging protocol, we require some participants to undergo an injection of intravenous gadolinium based contrast for a specific sequence (dynamic contrast enhanced MRI) for comparison to our novel water exchange method. At present, a recent (within 3 months) kidney function blood test is required for review. This is either taken from the participant's routine tests - if they have recently undergone blood tests for a clinical reason - or the study team complete the kidney function blood test. If the kidney function (eGFR) is >60, we administer contrast during the MRI scan. To reflect what is now becoming widespread research and clinical practice elsewhere, and in view of the current available evidence, we propose to lower the eGFR threshold to >40 to receive the intravenous contrast injection. This will have no implication on the existing resources in place at the participating organisations.

Applicability:	England	Wales	Scotland	Northern Ireland
Where are the participating NHS/HSC organisations located that will be affected by this change?*:	**Yes**	No	No	**Yes**
Will all participating NHS/HSC organisations be affected by this change, or only some? (**please note** that this answer may affect the categorisation for the change):	**All**		Some	

Add another change

Section 3: Declaration(s) and lock for submission

Declaration by the Sponsor or authorised delegate

- I confirm that the Sponsor takes responsibility for the completed amendment tool
- I confirm that I have been formally authorised by the Sponsor to complete the amendment tool on their behalf

Name [first name and surname]:*	Kina Bennett
Email address:*	kina.bennett@lthtr.nhs.uk

Lock for submission

Please note: This button will only become available when all mandatory (*) fields have been completed. When the button is available, clicking it will generate a locked PDF copy of the completed amendment tool which must be included in the amendment submission. Please ensure that the amendment tool is completed correctly before locking it for submission.

Lock for submission

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Section 4: Review bodies for the amendment

Please note: This section is for **information only**. Details in this section will complete automatically based on the options selected in Sections 1 and 2.

Review bodies																	
UK wide:						England and Wales:				Scotland:				Northern Ireland:			
									proval				g function				g function

	REC	Competent Authority MHRA - Medicines	Competent Authority MHRA - Devices	ARSAC	Radiation Assurance	UKSW Governance	REC (MCA)	CAG	HMPPS	HRA and HCRW Apr	REC (AWIA)	PBPP	SPS (RAEC)	National coordinating	HSC REC	HSC Data Guardians	Prisons	National coordinating	Category:
Change 1:						(Y)				(Y)								(Y)	C
Overall reviews for the amendment:																			
Full review:						N				N								N	
Notification only:						Y				Y								Y	
Overall amendment type:	Non-substantial, no study-wide review required																		
Overall Category:	C																		

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WEX-BRAIN study protocol

Calculate brain volume using Freesurfer

Required software / packages:

- Freesurfer
- MacOS

Method:

1) Obtain DICOM files from secure HDD or via the P:drive in Manchester
2) Using MRIcon – convert the DICOM file to NIfTI format
    a. Import > Convert DICOM to NIfTI > Select Folder To Convert
    b. Ensure that 'Output Format:' is set to 'Uncompressed NIfTI (.nii)'
    c. .nii files will ve saved into the same folder as the DICOM files
3) Navigate to folder, select .nii files for T1 and copy to the Freesurfer/7.3.2/subjects directory, rename to to 'sub_X'
4) Open 'Terminal'
5) Set up freesurfer…
    a. export FREESURFER_HOME=/Applications/freesurfer/7.3.2/
    b. source $FREESURFER_HOME/SetUpFreeSurfer.sh

```
Marks-MacBook-Pro:~ MarkMaskery$ export FREESURFER_HOME=/Applications/freesurfer
/7.3.2/
Marks-MacBook-Pro:~ MarkMaskery$ source $FREESURFER_HOME/SetUpFreeSurfer.sh
-------- freesurfer-darwin-macOS-7.3.2-20220804-6354275 --------
Setting up environment for FreeSurfer/FS-FAST (and FSL)
FREESURFER_HOME   /Applications/freesurfer/7.3.2/
FSFAST_HOME       /Applications/freesurfer/7.3.2//fsfast
FSF_OUTPUT_FORMAT nii.gz
SUBJECTS_DIR      /Applications/freesurfer/7.3.2//subjects
MNI_DIR           /Applications/freesurfer/7.3.2//mni
FSL_DIR           /Users/MarkMaskery/fsl
Marks-MacBook-Pro:~ MarkMaskery$
```

    c.
    d. Type: cd $SUBJECTS_DIR
    e. Type: recon-all -s sub-X -i sub_X.nii -all
    This will usually take approximately 12 hours to complete

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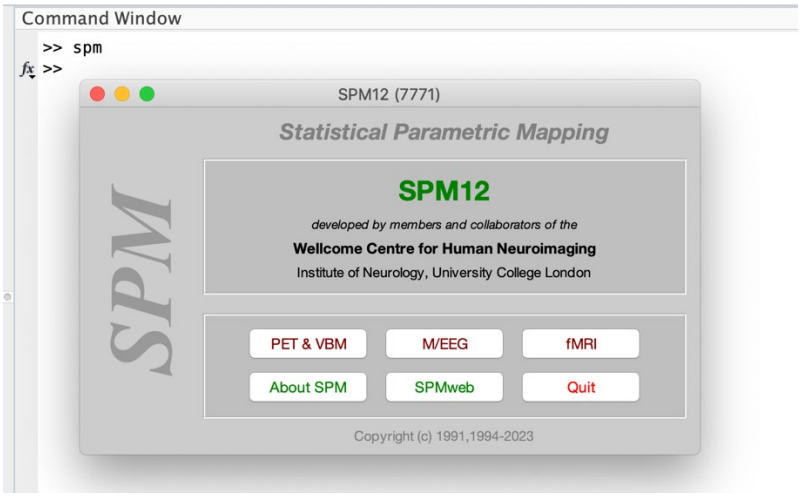
WEX-BRAIN study protocol

Calculate WMH volume using Lesion Segmentation Toolbox

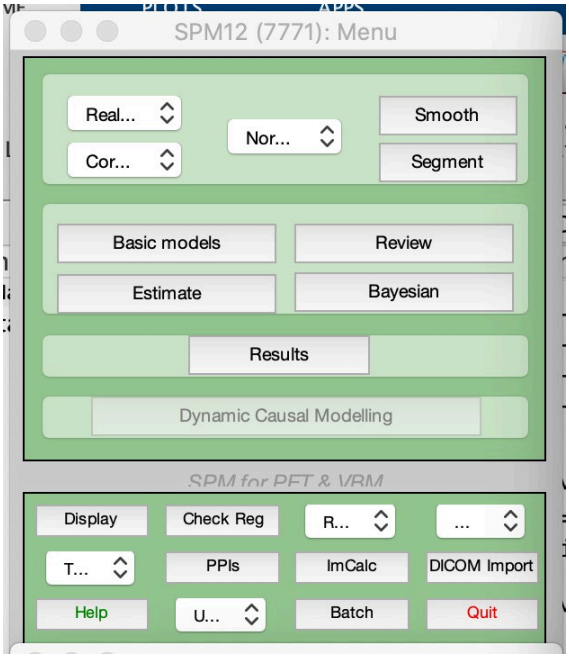
Required software / packages:

- Matlab + SPM + LST toolbox
- MRIcron

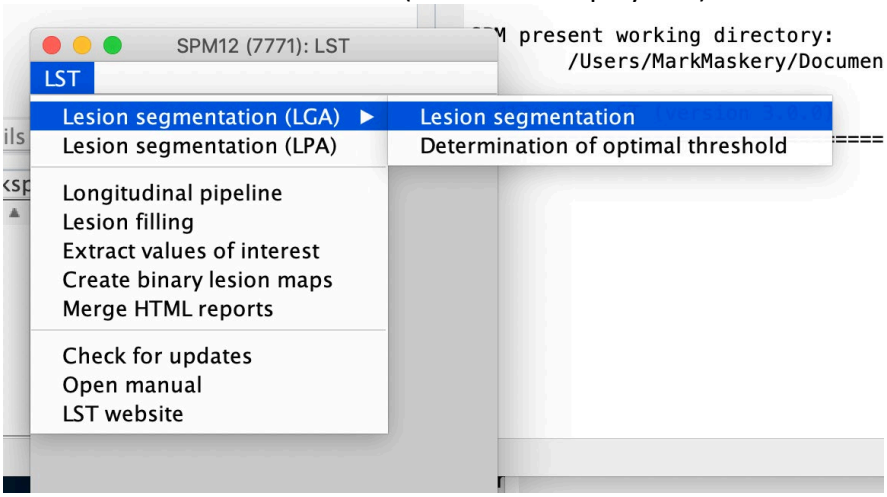
Method:

1) Obtain DICOM files from secure HDD or via the P:drive in Manchester
2) Using MRIcon – convert the DICOM file to NIfTI format
    a. Import > Convert DICOM to NIfTI > Select Folder To Convert
    b. Ensure that 'Output Format:' is set to 'Uncompressed NIfTI (.nii)'
    c. .nii files will ve saved into the same folder as the DICOM files
3) Navigate to folder, select .nii files for T1 and FLAIR, copy to separate folder to be used for WMH analysis
4) Launch Matlab
    a. Type 'spm' and hit Enter
    b. 

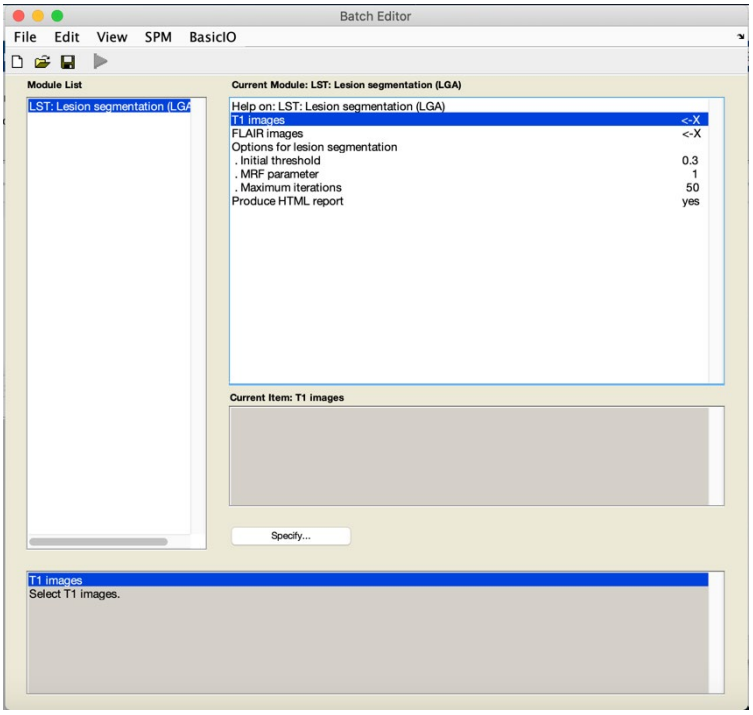
    c. Select 'PET & VBM'

d. 

e. Select ‘LST’ under the Toolbox (below the Display box)

f. 

g. LST > Lesion segmentation (LGA) > Lesion segmentation

h. 

i. Select ‘T1 images’ and navigate to the T1 .nii file
j. Select ‘FLAIR images’ and navigate to the FLAIR .nii file
k. Once files are assigned, click the ‘play’ (triangle) button at the top of the dialogue box to begin the process
l. This will usually take approximately 10 mins to complete, and will be signalled by ‘Done’ appearing in the Matlab Command Window.

To access the report, return to the folder which contains the T1 and FLAIR .nii files, you will find a new .html file called ‘report_LST_lga_0.3…’

- This will contain a line called ‘lesion volume’ and ‘number of lesions’ which should be recorded.

Completed.

T1-weighted & Freesurfer parcellation processing

1. Parcellation performed using Freesurfer
2. Brain extraction performed using FSL *BET* (Smith, HBM, 2002)
3. BBB-FEXI equilibrium scan registered to the T1w using FSL *epi_reg*; inverse of registration computed to obtain the T1-to-BBB-FEXI transformation
4. Inverse transformation applied to T1w to register T1w to BBB-FEXI (FSL *flirt*)
5. Inverse transformation used to propagate the aparc.a2009s+aseg parcellation to BBB-FEXI space, plus left/right WM/GM/CSF segmentations (FSL *flirt*)

T1 mapping

1. Each pre-contrast variable flip angle (VFA) spiral acquisition (flip angles = [2, 6, 10, 15] degrees) motion corrected using FSL *mcflirt* (Jenkinson et al., NeuroImage, 2002) (uses middle volume as the reference)
2. The 6 spirals of each VFA averaged using MRtrix3 *mrmath*
3. The averaged VFA registered to each other (flip angle = 2 as reference) using FSL *flirt*
4. T1 map calculated using Madym *madym_T1* (Berks et al., JOSS, 2021), including B1 correction
5. VFA with flip angle = 2 registered to BBB-FEXI equilibrium scan (FSL *flirt*)
6. Transformation used to propagate T1 map to BBB-FEXI space (FSL *flirt*)

T2 mapping (T2 map produced by scanner)

1. Echo 2 of the multi-echo acquisition registered to the BBB-FEXI equilibrium scan (FSL *flirt*)
2. Transformation used to propagate T2 map to BBB-FEXI space (FSL *flirt*)

FLAIR & WMH segmentation processing

1. FLAIR registered to BBB-FEXI equilibrium scan (FSL *flirt*)
2. Transformation used to propagate WMH segmentation to BBB-FEXI space (FSL *flirt*)
3. Propagated segmentation binarized using 0.3 (registration produces non-binary values)

DWI processing steps

1. Data denoised using MP-PCA method (Veraart et al., NeuroImage, 2016; Veraart et al., MRM, 2016; Cordero-Grande et al., NeuroImage, 2019) in MRtrix3 (Tournier et al., NeuroImage, 2019), *dwidenoise*, with a 3x3x3 kernel
2. Gibbs ringing corrected using method of sub-voxel shifts (Kellner et al., MRM, 2016), implemented in MRtrix3 (Tournier et al., NeuroImage, 2019), *mrdegibbs*
3. Susceptibility and eddy current correction performed using MRtrix 3 *dwifslpreproc* (relies on FSL *topup* and *eddy*)
4. Diffusion tensor fitted voxelwise using MRtrix3 *dwi2tensor*
5. Mean, radial and axial diffusivities (MD, RD, AD), and fractional anisotropy (FA) calculated using MRtrix3 *tensor2metric*
6. DWI registered to BBB-FEXI data using FSL *flirt*

7. All fits propagated to BBB-FEXI space (FSL *flirt*)

BBB-FEXI processing steps

1. DICOMS converted to nifti using dcm2niix
2. Each BBB-FEXI acquisition corrected independently for:
   a. Susceptibility distortions using FSL *topup* (Anderson et al., NeuroImage, 2003; Smith et al., NeuroImage, 2004)
   b. Eddy current distortions using FSL *eddy_correct* (Anderson et al., NeuroImage, 2003) (should also correct motion between volumes)
3. Each BBB-FEXI acquisition registered to the b=0 volume of the "equilibrium" acquisition (i.e. with tm=16ms and bf=0s/mm^2) using FSL *flirt* (Jenkinson et al., NeuroImage, 2002)
4. Each BBB-FEXI acquisition normalised to its respective b=0 volume
5. Each acquisition powder averaged (i.e. gradient directions averaged)
6. 2CM model fitted voxel-wise, using the T1/T2 maps (in BBB-FEXI space) where available, literature values where not
7. Regional/global values computed as median within each ROI (extreme fits within 1% of parameter constraints discarded)

References

1. Andersson J, Skare S, Ashburner J. How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging. Neuroimage. 2003;20:870-888. doi:10. 1016/S1053-8119(03)00336-7 53.
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3. Veraart, J.; Novikov, D.S.; Christiaens, D.; Ades-aron, B.; Sijbers, J. & Fieremans, E. Denoising of diffusion MRI using random matrix theory. NeuroImage, 2016, 142, 394-406, doi: 10.1016/j.neuroimage.2016.08.016
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6. **GENERAL MRTRIX REFERENCE**: Tournier, J.-D.; Smith, R. E.; Raffelt, D.; Tabbara, R.; Dhollander, T.; Pietsch, M.; Christiaens, D.; Jeurissen, B.; Yeh, C.-H. & Connelly, A. MRtrix3: A fast, flexible and open software framework for medical image processing and visualisation. NeuroImage, 2019, 202, 116137
7. Kellner, E; Dhital, B; Kiselev, V.G & Reisert, M. Gibbs-ringing artifact removal based on local subvoxel-shifts. Magnetic Resonance in Medicine, 2016, 76, 1574–1581
8. Jenkinson, M., Bannister, P., Brady, J. M. and Smith, S. M. Improved Optimisation for the Robust and Accurate Linear Registration and Motion Correction of Brain Images. NeuroImage, 17(2), 825-841, 2002.
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10. S.M. Smith. Fast robust automated brain extraction. Human Brain Mapping, 17(3):143-155, November 2002.