CLINICAL RELEVANCE OF CEREBRAL SMALL VESSEL DISEASES IN COGNITIVE IMPAIRMENT, NEURODEGENERATION AND STROKE

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Declaration:

I, Gargi Banerjee, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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PRIMARY SUPERVISOR: PROFESSOR DAVID J WERRING

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“The ideal man is he who in the midst of the greatest silence and solitude finds the intensest activity, and in the midst of the intensest activity, the silence and solitude of the desert. He has learnt the secret of restraint; he has controlled himself. He goes through the streets of a big city with all its traffic, and his mind is as calm as if he were in a cave where not a sound could reach him; but he is intensely working all the time.”

Swami Vivekananda, Karma-Yoga

Abstract

Cerebral small vessel diseases are common age-related processes associated with two important and highly prevalent clinical syndromes: stroke and dementia. Whilst our ability to define a specific small vessel disease neuropathologically (most usually post-mortem) is excellent, it is still difficult to reach a definitive diagnosis during life; there remains an unmet need to accurately classify and quantify different subtypes, especially if effective therapeutic trials are ever to be implemented. Thus, two important outstanding questions regarding cerebral small vessel diseases are: how do these processes contribute to cognitive decline and clinical prognosis, and how can we better recognise small vessel disease subtype and severity during life?

The programme of research described in this PhD thesis has three key aims. The first is to explore the role of cerebral small vessel diseases and their neuroimaging markers in specific patient populations. These include patients with cognitive impairment and dementia (a “memory clinic” population), patients with spontaneous (“primary”) intracerebral haemorrhage, and those presenting with cardioembolic ischaemic stroke or TIA (transient ischaemic attack). The second aim is to identify how and whether different small vessel disease subtypes (defined on the basis of intracerebral haemorrhage location) and their burden are associated with particular outcomes in patients with intracerebral haemorrhage. The outcomes of interest are recurrent intracerebral haemorrhage, subsequent cerebral ischaemic events (either ischaemic stroke or TIA), and death. The final aim is to present work from a prospective observational pilot study designed to identify new biomarkers for cerebral amyloid angiopathy, one of the most common cerebral small vessel diseases. In addition to describing the protocol and the recruitment process, results from body fluid analyses (cerebrospinal fluid and blood) and positron emission tomography (using the amyloid ligand ^{18}F-florbetapir) scanning will be presented. The implications and limitations of this work will then be discussed, together with proposals for future work in this field.

Impact statement

The data and knowledge described in this thesis has impact in a number of ways. Some of the projects presented have already been published in journals of clinical neurology, and this programme of research has contributed to a wider of body of work; a full list of manuscripts either published or submitted by the candidate during her time as a PhD candidate are listed in Appendix 2. Results from this thesis have also been presented at the European Stroke Organisation Conference in 2016, 2017 and 2018. Future dissemination of this work will include presentation of the results at regional, national and international conferences, and the publication of additional journal papers; another four manuscripts are in preparation on the basis of the projects presented here.

The academic impact of this work relates to the main results, which highlight the influence of cerebrovascular pathology in diverse populations. This finding is important for other researchers in this field, particularly those working on cognitive phenotypes, as it shows the complexity of the interaction between neurodegenerative and cerebrovascular pathologies. Pilot results from the BOCAA study will inform future projects in cerebral amyloid angiopathy (CAA) by providing much needed data on feasibility and sample size calculations. The body fluid findings, in particular the cerebrospinal fluid (CSF) findings, might have mechanistic implications for how and why CAA occurs, and should stimulate researchers to perform neuropathological and more complex CSF studies (for example, using stable-isotope labelling kinetics) in order to corroborate these results.

The clinical impact of this work falls into two broad categories. The first is its relevance for routine practice. The data within this thesis provides information on how structural imaging markers of cerebral small vessel disease, which are easy to identify on routine clinical MRI scans, can influence a patient's clinical outcome. This is therefore useful for

neurologists, geriatricians and general medical doctors as it provides guidance on identifying patients at greatest risk of adverse outcomes (including cognitive impairment, recurrent stroke, and death). Additionally, we show that the use of cognitive screening tools have relevance in day-to-day practice; this is particularly the case for the IQCODE, firstly as it has specific advantages in acute stroke populations (where an informant may be better placed to provide information than an acutely unwell patient), and secondly in view of its relationship with later functional outcome. The second way in which this work has clinical impact is by providing information for clinical triallists and the pharmaceutical industry. Our recruitment experience for the BOCAA study, together with our work on biomarkers in CAA, should inform future therapeutic trials, and hopefully allow development of a treatment for CAA, a condition for which there are currently no disease-modifying treatments.

The impact of this work will continue to be realised over the upcoming years as the remaining data for BOCAA are analysed, and with the ongoing dissemination of outputs, as described. This will form the basis for future research funding applications, which in turn will lead to further extension of the knowledge and expertise described within this thesis.

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Abbreviations

Aβ	Amyloid beta
ABIU	Acute brain injury unit
AD	Alzheimer's disease
ADC	Apparent diffusion coefficient
ADCI	Alzheimer's disease related cognitive impairment
ADNI	Alzheimer's disease neuroimaging initiative
AF	Atrial fibrillation
ANCA	Anti-neutrophil cytoplasmic antibody
APC	Admitted patient care
APOE	Apolipoprotein E
APP	Amyloid precursor protein
ARIA	Amyloid-related imaging abnormalities
BG-PVS	Basal ganglia perivascular space (MRI-visible)
BOCAA	Biomarkers and outcomes in CAA
BOLD	Blood oxygen level dependent
CAA	Cerebral amyloid angiopathy
CADASIL	Cerebral autosomal dominant arteriopathy with subcortical ischaemic strokes and leukoencephalopathy
CHARTS	Cerebral Haemorrhage Anatomical RaTing inStrument
CI	Confidence intervals
CMB	Cerebral microbleed
CROMIS-2	Clinical Relevance of Microbleeds in Stroke Study
cSAH	Convexity subarachnoid haemorrhage
CSF	Cerebrospinal fluid
CSO-PVS	Centrum semi-ovale perivascular space (MRI-visible)
cSS	Cortical superficial siderosis
CT	Computerised tomography
CV	Co-efficient of variance
DICOM	Digital Imaging and Communications in Medicine
DPA	Deep perforator arteriopathy
DWI	Diffusion weighted imaging
dWMH	Deep white matter hyperintensities
ECL	Electrochemiluminescence
ELISA	Enzyme-linked immunosorbent assay
FA	Fractional anisotropy

FLAIR	Fluid-attenuated inversion recovery
fMRI	Functional MRI
FWHM	Full-width at half-maximum (for haemodynamic response function)
GCA	Global cortical atrophy
GCS	Glasgow Coma Scale
GIF	Geodesic Information Flow
GP	General Practitioner
HASU	Hyperacute stroke unit
HCHWA-D	Hereditary Cerebral Haemorrhage with Amyloidosis-Dutch type
HES	Hospital episode statistics
HR	Hazard ratio
HV	Healthy volunteer
ICH	Intracerebral haemorrhage
IE	Ischaemic event
IQCODE	Informant Questionnaire for Cognitive Decline in the Elderly
IQR	Interquartile range
IV	Intraventricular
LRT	Likelihood ratio tests
MARS	Microbleed Anatomical Rating Scale
MBq	Megabecquerel
MCI	Mild cognitive impairment
MELAS	Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes
MMSE	Mini-mental state examination
MoCA	Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
mRS	Modified Rankin scale
MTA	Medial temporal atrophy
NFL	Neurofilament light
NHNN	National Hospital for Neurology and Neurosurgery, Queen Square
NHS	National Health Service
NIHSS	National Institutes of Health Stroke Scale
NOAC	Non-vitamin K oral anticoagulant
NODDI	Neurite orientation dispersion and density imaging
OR	Odds ratio

PET	Positron emission tomography
PiB	^{11}C-Pittsburgh B compound
PVC	Partial volume correction
PVS	MRI-visible perivascular space
pvWMH	Periventricular white matter hyperintensities.
rCBF	Regional cerebral blood flow
REC	Research ethics committee
ROI	Region of interest
SAH	Subarachnoid haemorrhage
sAPP	Soluble amyloid precursor protein
SD	Standard deviation
SHR	Subdistribution hazard ratio
SLE	Systemic lupus erythematosus
sTREM2	Soluble Triggering Receptor Expressed on Myeloid Cells 2
STRIVE	STandards for ReportIng Vascular changes on nEuroimaging
SUV	Standardised uptake value
SUVR	Standardised uptake value ratio
SVCI	Subcortical vascular cognitive impairment
SWI	Susceptibility weighted imaging
SVD	Small vessel disease
T2*-GRE	T2* gradient recalled echo
TE	Echo time
TFNE	Transient focal neurological episode
TIA	Transient ischaemic attack
TR	Repetition time
TREM2	Triggering Receptor Expressed on Myeloid Cells 2
UCLH	University College London Hospital
VOI	Volume of interest
VKA	Vitamin K anticoagulant
WMH	White matter hyperintensities

1 Introduction

Whilst cerebral small vessel diseases (SVDs) have been recognised for over 100 years (1) and described as “the most frequent pathological neurological process” (2), our understanding of how and why they cause disease remains limited. These common age-related processes are associated with two important and highly prevalent clinical syndromes: stroke and dementia (3). Both conditions have significant health and social impacts worldwide (4, 5), and thus SVDs provide an attractive potential target for reducing the burden of these diseases. However, whilst our ability to define a specific SVD neuropathologically (most usually post-mortem) is excellent (6), it is still difficult to reach a definitive SVD diagnosis during life. Recent advances in neuroimaging have shown great promise, but there remains an unmet need to accurately classify and quantify individual SVD subtypes, especially if effective therapeutic trials are ever to be implemented (7, 8). Furthermore, whilst there is a clear association between SVDs and cognitive impairment (2, 3), we still do not fully understand how SVDs disrupt cognition, whether all SVDs cause cognitive impairment in the same way, or if different pathological SVD processes have different effects. Thus, two important outstanding questions regarding SVD are: how do these processes contribute to cognitive decline and clinical prognosis, and how can we better recognise SVD subtype and severity in life?

The programme of research described in this PhD thesis has three broad aims:

1. To explore the role of SVDs and their neuroimaging markers in specific patient populations
2. To investigate how and whether SVD subtype (defined by intracerebral haemorrhage location) and burden is associated with outcomes in patients with intracerebral haemorrhage (ICH)
3. To identify new biomarkers for sporadic amyloid-β cerebral amyloid angiopathy (CAA), one of the most frequently observed SVDs

1.1 What are SVDs, and how are they classified?

SVDs are pathological processes that affect the small arteries, arterioles, capillaries and small veins of the brain; the size of affected vessels can range from a few hundred microns to approximately a millimetre (2, 3). These processes result in cerebral damage via incomplete and complete necrosis (due to chronic hypoperfusion or total vessel occlusion, or both), blood brain barrier disruption, local inflammatory processes and oligodendrocyte loss (2, 3).

Whilst there are many SVD subtypes (Table 1.1.1), types 1 and 2 are by far the most common (2, 3). Type 1 SVD has a number of names, including arteriolosclerosis, age-related small vessel disease, vascular risk factor associated small vessel disease, hypertensive arteriopathy and deep perforator arteriopathy (DPA, which will be used in this thesis)(2, 3). This variety of terms reflects the association of this SVD with hypertension, diabetes and ageing, and its associations with systemic manifestations of small vessel damage, such as microvascular renal and retinal dysfunction (2, 3). Neuropathological observations in DPA include small vessel segmental arterial disorganization, fibrinoid degeneration, lipohyalinosis and evidence of microatheroma (3, 9). DPA is thought to be responsible for many clinically “silent” cerebral ischaemic lesions and all lacunar infarcts, as well as “deep” (occurring in the basal ganglia, thalamus and brainstem) ICH (3, 9-13).

Type 2 SVD is cerebral amyloid angiopathy (CAA), which is characterised by the deposition of amyloid in the walls of cortical and leptomeningeal arterioles and capillaries of the brain (3, 14, 15). Although there are many potentially causative amyloid proteins, sporadic amyloid-β (Aβ) CAA is by far the most common (subsequent reference to CAA in this thesis will refer to this sporadic type) (3, 16). Pathologically, the deposits are first seen in the abluminal tunica media, with progression eventually resulting in panmural

accumulation (3, 14). This is associated with smooth muscle cell degeneration, thickening of the vessel wall and narrowing of its lumen, and eventually concentric splitting of the vessel wall ("double-barrelling"), microaneurysm formation, and perivascular microhaemorrhage (3, 17).

Table 1.1.1: Classification of small vessel diseases

Adapted from (2) and (3).

Subtype	Name	Examples (where relevant)
1	DPA	
2	CAA (hereditary and sporadic)	
3	Inherited / genetic small vessel diseases	CADASIL MELAS Fabry disease
4	Inflammatory / immunologically mediated small vessel diseases	Nervous system vasculitides (e.g. SLE, scleroderma, ANCA-associated) Nervous system vasculitides secondary to infection
5	Venous collagenosis	
6	Other	Post-radiation angiopathy

1.2 Why do we need to differentiate between SVD subtypes?

The primary motivation for differentiating between DPA and CAA is clinical. The association between CAA and recurrent lobar ICH is well recognised (14, 18), and so the use of statins (19), anti-thrombotics (20) and anticoagulants (21) (as treatments that might increase the baseline risk of ICH) in this patient group is generally not advised. In contrast, DPA is associated with cardiovascular risk factors and lacunar infarction (2), and thus might present a greater ischaemic risk together with lower rates of ICH recurrence. Given this, anti-thrombotics and statins are more likely to be indicated in DPA, and these agents might not increase the risk of subsequent ICH to the same extent as in CAA. However, this view of "bleeding-prone" CAA (2, 22) versus "ischaemia-prone" DPA is almost certainly an oversimplification. A recent individual patient data meta-analysis (23) following patients with atrial fibrillation (AF) who resumed treatment with oral anticoagulant therapy following spontaneous ICH, found that resumption was associated with reduced mortality and all-cause stroke incidence, as well as more favourable outcomes, at 1 year. Moreover, this effect was also observed in patients with

lobar ICH (usually associated with CAA). This might suggest that, in patients with lobar ICH at elevated thrombotic risk (e.g. AF), the “early” (i.e. up to 1 year) risk of ischaemic events may be underestimated and that they might benefit from anticoagulation during this period. However, the data on later stroke events in patients with ICH is limited (20, 24-26), as ICH is associated with high rates of mortality (survival at 1 year is estimated to be 46%, with 5 year survival only 29% (20)), with data on outcomes in the longer term (beyond 1 year) being particularly scarce.

A further reason for improved methods for identifying SVD subtype during life is that, given the fundamental differences in the underlying pathophysiology of these two SVDs, accurate diagnostic identification is likely to be essential for future therapeutic trials. Moreover, given that DPA and CAA are both age-related processes, being better able to distinguish them from one another would improve our understanding of their relative roles as co-pathologies in conditions where multiple pathologies may contribute to eventual cognitive decline and dementia (in a manner analagous to that demonstrated in post mortem neuropathological studies (27)).

1.3 How can we differentiate between CAA and DPA at present?

All of the non-pathological methods currently in use to distinguish between CAA and DPA rely on the observation that these two SVDs predominantly affect blood vessels with different anatomical distributions (Figure 1.3.1); it is worth noting that whilst this anatomical division has a pathological basis, it is likely to be something of a generalisation in a clinical context (28-30). The most frequent clinical use of this principle is in patients with ICH, where lobar ICH is attributed to CAA and deep ICH to DPA (31); it is now increasingly used for other structural neuroimaging markers of SVD. As an example, cortical superficial siderosis (cSS) is believed to occur due to haemosiderin deposition in the subpial space and underlying cortical convexities following an acute convexity subarachnoid haemorrhage (cSAH) (32). The location of this haemorrhagic

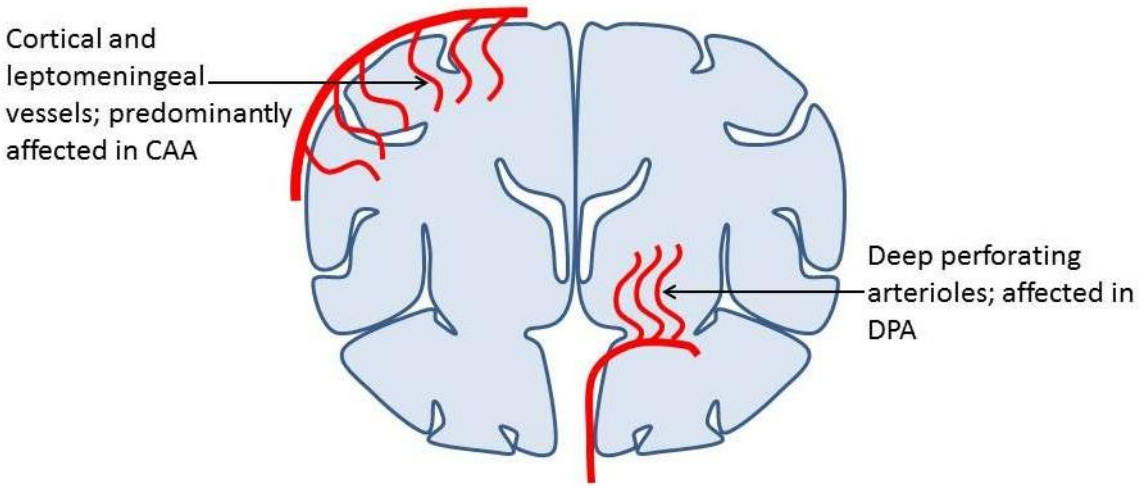
phenomenon (i.e. cortical) is in keeping with the known anatomical distribution of CAA, and is now recognised as a key imaging feature of the disease (32).

This approach of anatomically categorising imaging features as being associated with either CAA or DPA is now being applied to more widely distributed structural markers. Cerebral microbleeds (CMB) are small hypointense lesions observed on paramagnetic-sensitive MR sequences (3, 33), and are associated with CAA when found in lobar or cortico-subcortical regions, whereas those that are deep or infratentorial are associated with DPA (3, 34, 35). MRI-visible perivascular spaces (PVS) are thought to be abnormal enlargements of the perivascular space, a component of the neurovascular unit (36). The exact location of the perivascular space has been variously described; it has been both defined as the potential space between the outer aspect of a vessel wall and the brain parenchyma (3, 7, 37, 38), and as being within the vessel wall itself, within the tissue space of the tunica adventitia and the basement membrane surrounding smooth muscle cells in the tunia media of arteries and arterioles (36). Some of this variability is likely to reflect the differences in vessel structure between arteries, capillaries and veins, as well as differences between arteries and arterioles of different sizes (39). The nature of the perivascular space also varies with the anatomical location of arteries, with variations in size (between cortical arteries and those within the subarachnoid space) and the number of surrounding leptomenigeal layers (for arteries in the basal ganglia) described (39). In the context of small vessel disease, PVS in the basal ganglia (BG-PVS) have been associated with markers of DPA, whereas PVS in the white matter centrum semi-ovale (CSO-PVS) are associated with cerebral Aβ pathologies - both Alzheimer's disease (AD) and CAA (40-42).

Whilst this topographical dichotomy has the advantage of being simple and having a biological basis, it has some intrinsic limitations. An association between deep ICH and lobar CMBs has been described (43, 44), raising questions about whether lobar CMBs are exclusively a feature of CAA. Moreover, a significant proportion of ICH patients (up

to a quarter (45)) have “mixed” disease (i.e. features of both CAA and DPA), which perhaps is unsurprising given that both SVDs are common and age-related. We currently regard this “mixed” group as non-CAA, primarily because although DPA may be associated with lobar MB, the obverse has not been found; however, recent evidence suggests that this group may still have a significant Aβ burden, and the ratio of lobar to deep CMB may be more effective at determining the predominant SVD (46). PVS are similarly limited, as in some cases the frequency and severity of BG-PVS and CSO-PVS are correlated with each other (47). These limitations highlight the need for better biomarkers, using neuroimaging and other modalities, for cerebral SVDs.

Figure 1.3.1: Schematic demonstrating vessels affected in CAA and DPA



1.4 Identifying new biomarkers for cerebral SVDs: the argument for prioritising CAA

There are four arguments for prioritising CAA over other SVDs when it comes to biomarker development:

1) CAA has the greatest morbidity and mortality of the SVDs, given its association with recurrent ICH (15). This is based upon its association with lobar ICH (a recent meta-analysis quotes an OR of 2.21, 95% CI 1.09 to 4.45 (48)), the subtype of ICH which is more likely to recur (annual recurrence rate 2.5 to 14.3% compared with 1.3 to 2.9% for non-lobar ICH) (15, 20). Given that the estimated 1 year survival for ICH is 46% (20), and that more than 60% of ICH survivors are dependent due to severe physical or cognitive impairments (49), the burden of disease attributable to CAA is likely to be significant (15).

2) The ability to identify and isolate the impact of CAA in patients with multiple neuropathologies has mechanistic importance. As well as a contribution to cognitive impairment in patients with ICH, CAA appears to have an independent effect on cognition in patients with AD, which is of interest because AD and CAA frequently coexist (50, 51). Recent neuropathological work has demonstrated that CAA makes an independent cognitive contribution to AD dementia, even after adjusting for other age-related pathologies including AD pathology (50). There is evidence that patients with familial AD develop white matter hyperintensities (WMH), a recognised feature of CAA (52), up to 6 years before their estimated symptom onset, and that WMH may be a “core feature” of familial AD (53); the predominantly parietal and occipital distribution of these WMH are consistent with the distribution seen in sporadic CAA (53, 54). Moreover, cortical atrophy, an imaging finding previously felt to be primarily representative of AD pathology, has been shown to occur in CAA even in the absence of coexistent AD pathology (55). Patients with both strictly lobar CMBs and AD demonstrate more grey matter atrophy and greater reductions in glucose metabolism than those with AD and without strictly lobar CMBs (56). These findings together suggest that certain clinical and radiological features that have previously been thought to exclusively represent AD pathology might in fact be manifestations

of CAA (at least in part), and it could be argued that future treatment strategies for AD that do not consider the impact of CAA might thus be less effective (50).

3) CAA shares a core pathological element (the Aβ protein) with AD, which has allowed CAA research to take advantage of the rapid technological advances that have taken place in the AD field, for example the development of amyloid-PET ligands and the identification of cerebrospinal fluid (CSF) biomarkers. This opportune connection might also expedite the identification of an effective treatment for CAA before other SVDs; identifying biomarkers that can be used as outcome markers in clinical trials is therefore a priority (8).

4) Although the current diagnostic criteria are not without limitations, CAA has a further advantage over other SVDs in that it can be reliability identified during life with good specificity (81.2%) and sensitivity (94.7%) using the modified Boston criteria (57). Newer CT-based criteria (the Edinburgh criteria (58) are yet to be fully validated, but raise the possibility of reliably identifying CAA without the need for MRI; this is likely to increase the number of patients identified as having the disease.

1.5 What are our current biomarkers for CAA?

1.5.1 Imaging

Neuroimaging remains central to the diagnosis of CAA; the focus here has been on the presence of haemorrhagic manifestations of the disease on MRI (Figure 1.5.1), specifically lobar ICH (or “macro” haemorrhage), strictly lobar CMB (“micro” haemorrhage) and cSS, all of which are included in the now established MRI-based modified Boston diagnostic criteria (57, 59). The more recent Edinburgh diagnostic criteria are also imaging (CT) based; two of the three criteria are features of lobar haemorrhage (“finger-like projections” and subarachnoid extension) (58). Whilst multiple

lobar ICH remains the strongest indicator for CAA, they are a late feature of the disease and may not be a practical outcome marker for clinical trials (8). Acute cSAH has been shown to evolve over weeks to months (32) into cSS, a recognised MRI marker of CAA (60-62). Clinically, both acute cSAH (63, 64) and cSS (61) are associated with transient focal neurological episodes (TFNE; Figure 1.5.2); cSS is also associated with an increased risk of ICH (65-70), including early recurrent ICH (71). It is likely to be a marker of severe CAA (32), and there is some evidence that cSS is associated with higher levels of amyloid deposition, as measured by amyloid-PET (72, 73). Finally, whilst strictly lobar CMBs remain important for diagnosis, recent work has found that whilst they were strongly predictive of CAA in a hospital cohort, this was not the case in a healthy community population (74). This has highlighted the importance of identifying new imaging markers for CAA that can further improve the specificity and sensitivity of diagnosis (8, 75), particularly in cohorts without ICH.

In the last five years new “non-haemorrhagic” (8) structural and functional imaging markers for CAA have emerged (Table 1.5.1). Whilst many of these markers are predominantly used in a research capacity, with use limited to academic medical centres, two of them (MRI-visible perivascular spaces and cortical microinfarcts) are quantifiable on routine 3-Tesla imaging. MRI-visible perivascular spaces, described earlier, are hypothesised to result from enlargement of the potential space either within or outside a blood vessel wall, possibly secondary to impaired interstitial fluid drainage (7, 37). As described above, CSO-PVS are associated with cerebral Aβ (AD and CAA) pathologies (40-42), and it is hypothesised that this occurs as a consequence of failed Aβ clearance (37, 76, 77). Cortical microinfarcts, initially identified pathologically using brain tissue and later with 7-Tesla imaging (78, 79), have now been identified using 3-Tesla MRI (80-84). They are of interest as they appear to correlate closely with cognitive performance (78, 79), and might be of particular importance in CAA-related cognitive impairment (85).

Establishing CAA severity using neuroimaging remains a challenge; a composite score has been proposed (86), which aims to estimate the overall pathological “burden” of CAA by combining key imaging markers of CAA, with some preliminary pathological verification of the concept. However, as mentioned in the previous paragraphs, the main limitation of the currently available imaging biomarkers for CAA is that the haemorrhagic markers are often late features of the disease, and many of the non-haemorrhagic markers are not specific to CAA (8). One way in which the sensitivity and specificity of the current or any future imaging measures could be improved is by using them in combination with another modality, for example body fluid markers or neuropsychological measures.

Figure 1.5.1: Haemorrhagic manifestations of CAA

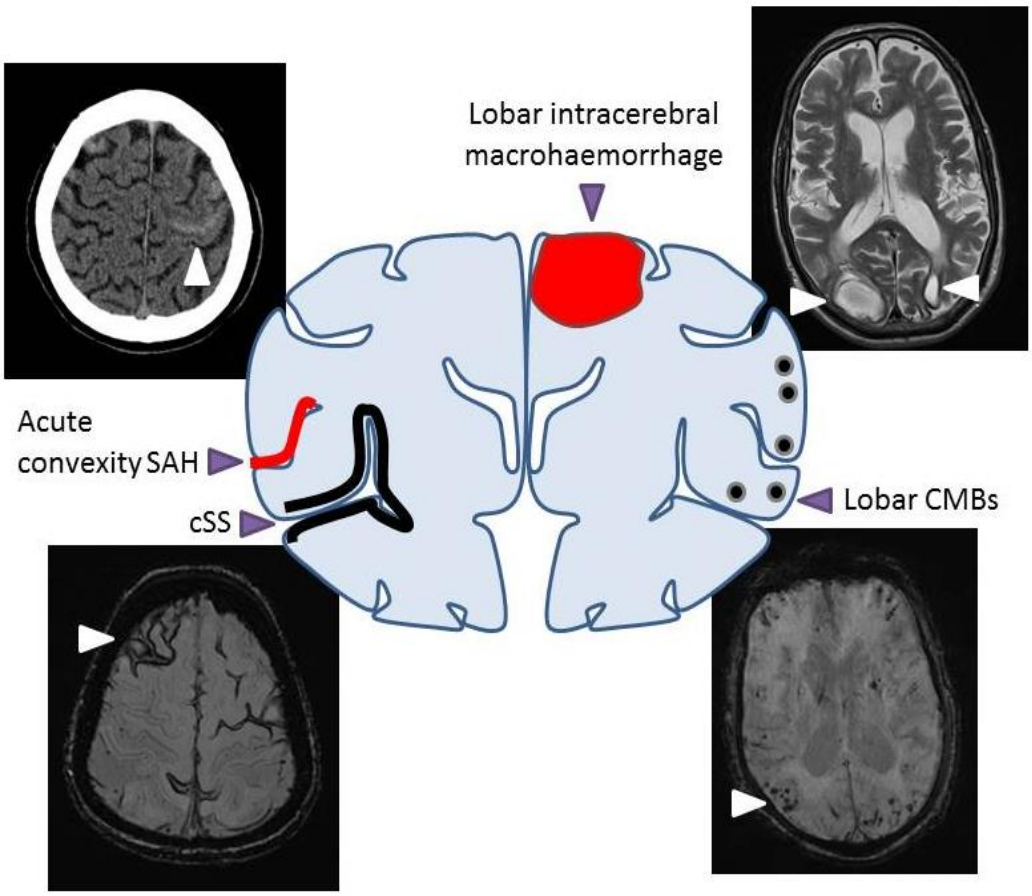


Figure 1.5.2: Imaging findings in CAA-associated TFNE
Figure and caption taken from (87); both were originally produced by the candidate for publication.
These images from a 76-year-old patient who presented with migratory left-sided sensory symptoms consistent with CAA-associated TFNE. The original CT (A) shows a hyperdense area in keeping with an acute cSAH (arrow). Three months later, the patient had a similar episode; repeat CT (B) at this time demonstrated another acute cSAH nearby (arrow). Subsequent susceptibility weighted MRI (C and D) showed widespread disseminated cSS affecting the right hemisphere (arrowheads).

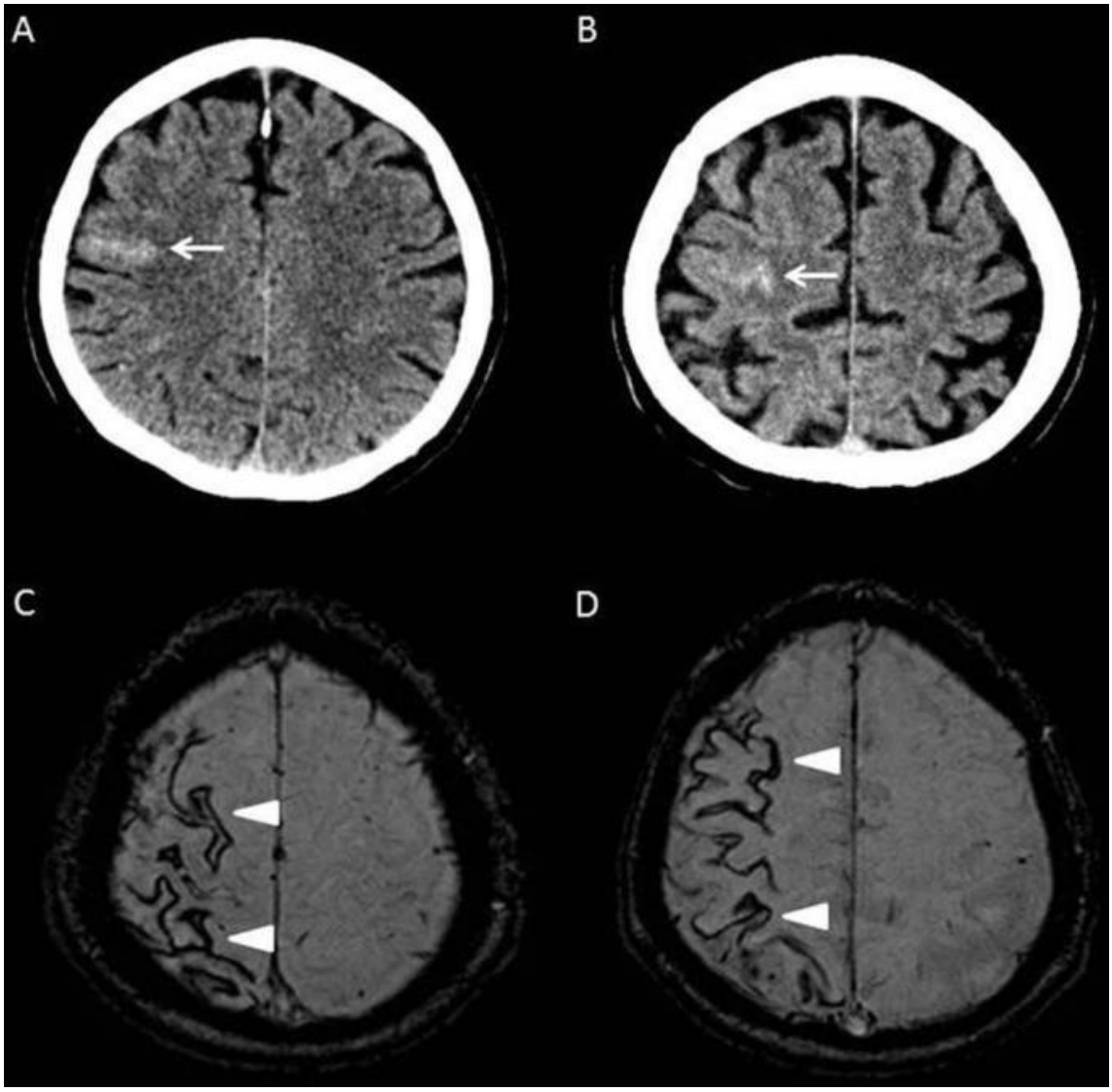


Table 1.5.1: Summary of potential new non-haemorrhagic structural and functional imaging markers for sporadic CAA
Adapted from (87); original table produced by the candidate for this publication.

IMAGING MARKER	EVIDENCE OF POTENTIAL AS A BIOMARKER IN CAA	LIMITATIONS
MRI visible perivascular spaces in the centrum semi-ovale (CSO-PVS)	• Severe or high grade CSO-PVS commonly observed in CAA (42, 88-92) • Higher CSO-PVS volume is associated with sporadic CAA (93) • Pilot data show that, in those with CAA, CSO-PVS severity is associated with Aβ burden (as measured by PiB) (94)	• Non-specific (age-related); present in a number of other conditions (47)
Cortical atrophy	• Thinner cortices observed in those with sporadic CAA compared with healthy controls; occipital, temporal, posterior parietal and medial frontal areas affected (55) • In patients with probable CAA and cognitive impairment, different profiles of atrophy were observed for patients with cSS (precuneus, posterior cingulate, parieto-temporal, superior frontal and medial temporal regions) and those without (parieto-temporal, superior frontal and precentral regions) (95)	• Difficult to differentiate between atrophy secondary to parenchymal Aβ and that due to vascular Aβ in sporadic CAA
Cortical microinfarcts	• Small asymptomatic DWI lesions have been detected in patients with CAA (96) • Microinfarcts have been identified on post mortem 7-Tesla MRI in patients with CAA (97) • In a recent 3-Tesla MRI in-vivo study, patients with CAA had more cortical microinfarcts that patients with AD and healthy controls, and new cortical microinfarcts were observed at 1 year follow up (84)	• Microinfarcts remain difficult to identify *in vivo*; even values obtained using 7-Tesla MRI are likely to be underestimates (78)
Functional MRI	• Patients with CAA have abnormal BOLD responses to a visual stimulus (alternating checkerboard), with reduced response amplitude and prolonged time both to peak and to baseline (98, 99) • Those with CAA show a decline in this BOLD amplitude that is detectable at 1 year; longitudinal difference in BOLD amplitudes was significantly lower in CAA compared to controls (100) • Haemodynamic response functions for primary visual and primary motor cortex show abnormalities in patients with CAA, with changes in time to peak,	• Clinical implications of this remain unclear; due to technical factors this is (at present) predominantly a research tool, and limited to academic medical centres

	full-width at half-maximum (FWHM) and area under the curve measures compared with healthy controls; visual cortex FWHM was associated with CMB count (101) • Potentially of interest as a surrogate marker of vascular health in clinical trials	
Network measures	• Lower global efficiency of brain network in those with CAA; occipital, parietal, and posterior temporal lobes most effected (102) • Reduced efficiency correlated with Aβ burden (as measured by PiB), as well as with impaired executive function and processing speed (102) • Increasing CAA severity, as measured by a composite neuroimaging CAA score, was associated with reductions in global network efficiency (103) • Global efficiency slows a longitudinal decline with time (mean follow up 1.3 years) in those with CAA, and is associated with deteriorating executive function (104) • The decline in posterior network connectivity observed over time is associated with occipital cortical atrophy (105)	• Difficult to differentiate between network effects of parenchymal Aβ versus vascular Aβ in sporadic CAA • Mainly a research tool limited to academic medical centres
Amyloid-PET imaging using ^{11}C-PiB-PET and ^{18}F compounds	• Recent meta-analysis (7 studies) found that amyloid-PET had “moderate to good diagnostic accuracy” for CAA, with overall pooled sensitivity 79% (95% CI 62 to 89%) and specificity 78% (95% CI 67 to 86%) (106) • In those with CAA, regions with high PiB retention area have been associated with subsequent haemorrhage (107) • Although PiB-PET may not reliably distinguish between patients and age matched controls (108), early phase (1–6min) uptake can do this (109) • The occipital/posterior cingulate ratio of PiB uptake is different for those with CAA versus those with AD (109); a recent meta-analysis (7 studies) found that occipital/global ratio may be able to differentiate between CAA and AD (110) • PiB-PET and ^{18}F-florbetapir binding are able to distinguish between CAA-associated ICH and hypertension-associated ICH (111, 112)	• Amyloid-PET unable to differentiate between vascular and parenchymal Aβ • Diagnostic accuracy for CAA seems limited • Few data on change over time in CAA

1.5.2 Body fluid markers

Most of the work on potential body fluid biomarkers in CAA has been using CSF; as mentioned previously, this is in all likelihood due to the successful development of CSF biomarkers in AD (113, 114). The study of CSF in CAA might provide an opportunity to better understand one of the major outstanding questions in CAA, namely why deposition is predominantly (and in some cases exclusively) vascular, in contrast to the parenchymal deposition observed in AD. Advances in our understanding of protein clearance and the fluid compartments of the brain have led to a hypothesis that CAA results from failures of Aβ clearance (76). This hypothesis is supported by the observation that AD patients treated with anti-Aβ immunotherapies develop CAA-like imaging features, which themselves are associated with successful Aβ clearance (115-117). These imaging features (ARIA; amyloid-related imaging abnormalities) resemble those observed in the inflammatory variant of CAA (118), where patients spontaneously develop autoantibodies to the Aβ protein (119); this raises the intriguing possibility that CAA is a manifestation of attempted “physiological” Aβ clearance. Whilst CAA related inflammation usually presents with serious neurological symptoms (including seizures, encephalopathy and focal neurological deficits), patients with minimal or no symptoms have now also been described (120). Moreover, there is recent neuropathological evidence that sites of microhaemorrhage are associated with lower levels of vascular Aβ deposition and less severe CAA (121), further supporting this hypothesis. As a consequence, an improved understanding of the CSF measures in CAA might have both mechanistic and diagnostic implications.

The major CSF and blood biomarker findings for CAA to date are summarised in Table 1.5.2. The perturbation of CSF Aβ-40 and Aβ-42 in presymptomatic carriers of HCHWA-D (hereditary cerebral haemorrhage with amyloidosis - Dutch type), an inherited form of Aβ CAA caused by a mutation in the amyloid precursor protein (APP) gene, highlights

its potential as an early biomarker for the sporadic form of the disease (122). Whilst there is some variability in the data, CAA is generally associated with lower CSF Aβ-40 and Aβ-42 than both control subjects and patients with AD; CAA patients also have a higher Aβ-40: Aβ-42 ratio than AD patients. CSF tau markers in CAA appear to fill an intermediate position, with levels higher than those observed in controls but lower than those seen in AD. The data for blood biomarkers of CAA are more limited, and restricted to Aβ-40 and Aβ-42 only.

1.5.3 Neuropsychology

The association between CAA and cognitive impairment has been recognised for some time; CAA is associated with an increased risk of developing dementia in ICH survivors (123, 124), and the prevalence of mild cognitive impairment in patients with CAA was 79% in one study (125). Data from neuropathological studies has demonstrated that CAA as a pathology makes an independent contribution to cognitive performance (27, 50, 85, 126), and it has been argued that CAA should be considered as a neurodegenerative condition (127). However, the neuropsychological profile of CAA is yet to be exploited as a disease biomarker. CAA has been associated with an accelerated decline in global cognition, as well as specific deficits in processing speed, executive function, language skills, visuospatial functioning and episodic memory (50, 102, 125, 128-132), but it is unclear which of these deficits is first to manifest. Additionally, the extent to which this data is confounded by the presence of structural damage due to ICH and pre-existing cognitive impairment remains uncertain. The association between ICH and later cognitive impairment is well recognised (123, 133, 134), but there is evidence that CAA has an independent impact on cognition; in patients with CAA and without ICH, increasing disease severity (as measured by a composite CAA score), was associated with the development of dementia (135).

Finally, there have been case reports of CAA presenting with neuropsychiatric symptoms, including delirium, depression, and personality change (136-138); in patients with AD, and the neuropathological presence of advanced CAA was associated with severe psychotic symptoms during life (139). The “vascular depression hypothesis” (140) proposes that SVDs might contribute to depression, and there is evidence for an association between structural imaging markers of SVD and depressive symptoms (141-147). In particular, CMBs have been associated with depressive symptoms in patients with AD (148) as well as the general population (149). Further work is needed to establish whether a similar mechanism results in the neuropsychiatric symptoms that have been observed in CAA.

Table 1.5.2: Fluid biomarkers in CAA

		Hereditary CAA	Sporadic CAA	Memory Clinic Populations
Amyloid markers (Aβ-40 and Aβ-42)	CSF	CSF Aβ-40 and Aβ-42 are reduced in presymptomatic and symptomatic carriers of the HCHWA-D APP mutation compared with controls; reductions in CSF Aβ-40 are associated with imaging features of CAA (higher number of lobar CMBs, presence of cSS, increasing WMH volume) (122).	Patients with probable CAA have lower Aβ-40 and Aβ-42 than both control subjects and those with AD (150, 151). In one study, Aβ-42 levels were lower in CAA than controls, but higher than patients with AD; Aβ-40 levels were lower than AD patients, and non-significantly lower than controls (152). Patients with cSS have lower Aβ-42 than controls, and lower Aβ-40 than those with AD (153).	The presence of cortical CMBs is associated with reduced CSF Aβ-40 and Aβ-42 in patients with AD (154). The presence of multiple CMBs (>8) in AD patients is associated with a lower CSF Aβ-42 than AD patients without any CMBs (155). Those with microbleeds, particularly those in a distribution in keeping with CAA, have lower CSF Aβ-42 levels (156-158). cSS is associated with reduced CSF Aβ-42 in a memory clinic population (159)
	Blood	Carriers of the HCHWA-D APP mutation have lower plasma Aβ-42 than non-carriers (160).	Patients with probable CAA have higher plasma Aβ-40 and Aβ-42 compared with controls (161).	-
CSF tau markers (total tau, phospho-tau)		Symptomatic carriers of the HCHWA-D APP mutation have lower CSF phospho-tau levels compared with controls; presymptomatic mutation carriers showed no difference in CSF total tau or phospho-tau compared with either controls or symptomatic carriers (122).	Patients with probable CAA have CSF total tau and phospho-tau levels that are higher than controls, but lower than patients with AD (150, 151). Those with cSS have higher total tau than controls and those with CAA associated lobar ICH, but lower total tau and phospho-tau than those with AD (153). Total tau and phospho-tau levels in patients with CAA are higher than those in controls, but lower than those in AD (152).	AD patients with multiple CMBs (>8) had higher CSF total tau and phospho-tau levels than AD patients without any CMBs (155).

2 Small vessel diseases and their structural markers in different patient populations

Sections 2.1 and 2.2 are taken from published work by the candidate (3, 162).

2.1 Vascular contributions to cognitive impairment and dementia - why do they matter?

There is no doubt that dementia is a growing concern globally; given the aging population, the number of people affected has been projected to double over the next two decades, with the economic impact expected to rise over 85% (163). The vascular cognitive impairment and vascular dementia concepts are of clinical and research importance because vascular factors might be treatable, and thus providing a potential strategy to reduce disease progression. Indeed, recent data suggest that the “dementia epidemic” has not yet occurred to the extent predicted (164), which might be due in part to the improved treatment of modifiable vascular risk factors, for example hypertension or dyslipidaemia (165).

2.2 Structural markers of small vessel disease: the role of neuroimaging

The advent of brain imaging has revolutionised the identification and quantification of cerebral SVDs during life. Markers now considered “classical” for SVD (lacunar infarcts, WMH, brain atrophy) were identified in the 1970s and 1980s (initially on CT) (3), and their associations with cognitive impairment have been extensively described (166-178). More recently identified markers, including CMBs, cSS and PVS, have shown associations with cognitive impairment, but these associations are not as consistently reported as those for the earlier “classical” markers (3). Reasons for this may include

differences in definition and rating methods, imaging acquisition and the MR field strengths used; in view to this, attempts have been made to standardise the manner in which these SVD markers are quantified (7). Additionally, many newer markers are not specific for SVD (this is particularly the case for PVS (3)), and different markers (and distributions) are likely to reflect different SVD subtypes (as described in Section 1.3), not all of which may be present in a single individual or disease population.

Recently, there has been interest in combining separate SVD imaging markers in order to better reflect overall burden; the "total SVD score" (179) (which concentrates on imaging features of DPA) has shown associations with a number of clinical measures including cognitive performance (180-183), recurrent stroke (both ischaemic and haemorrhagic) (184), gait and balance measures (185, 186) and mortality (187). These studies have predominantly focussed on populations with higher cardiovascular risk, for example those with a history of hypertension, previous TIA or ischaemic stroke, but there are also studies in the healthy elderly (aged over 60 years) (181, 183, 188). A similar composite score for CAA has now also been developed (86), which might allow CAA severity to be quantified for the first time. This score has shown correlations (in patients with CAA) with TFNE (189), incident dementia risk (135), ICH recurrence (190) and reductions in global network efficiency (103), in addition to the neuropathological severity of CAA-related changes (86). These scores provide a new method for estimating SVD impact in an individual, but concerns remain about the specificity of the SVD score (two of the component markers are also associated with CAA). Additionally, the CAA score is weighted such that lobar CMB and cSS, which are also features of the modified Boston criteria, score more highly than the other components; given this, the utility of lower values of the CAA score is not clear, and it is possible that the associations observed are driven by the binary presence or absence of CAA. The application of these promising scores in broader populations (and in the case of the CAA score, in populations who do not meet the modified Boston CAA) would help to confirm their value.

This section describes four projects investigating the presence and associations of structural markers of SVD in different patient populations. The first project considers the associations between diagnosis and PVS location in a memory clinic population; the second reviews the associations of these markers with cognitive impairment prior to ICH; the third and fourth consider the associations of these markers in patients with ischaemic stroke or TIA and AF, looking at pre-event and 12 month cognitive performance respectively.

2.3 General Methods

This section is adapted from published work by the candidate (38, 191).

Rating of all SVD structural markers was performed based on the following methods, unless stated otherwise in the text. In all cases, rating was completed in accordance with the STRIVE (STandards for ReportIng Vascular changes on nEuroimaging) consensus criteria (7), and the rater was blinded to all clinical information.

2.3.1 MRI-visible perivascular spaces

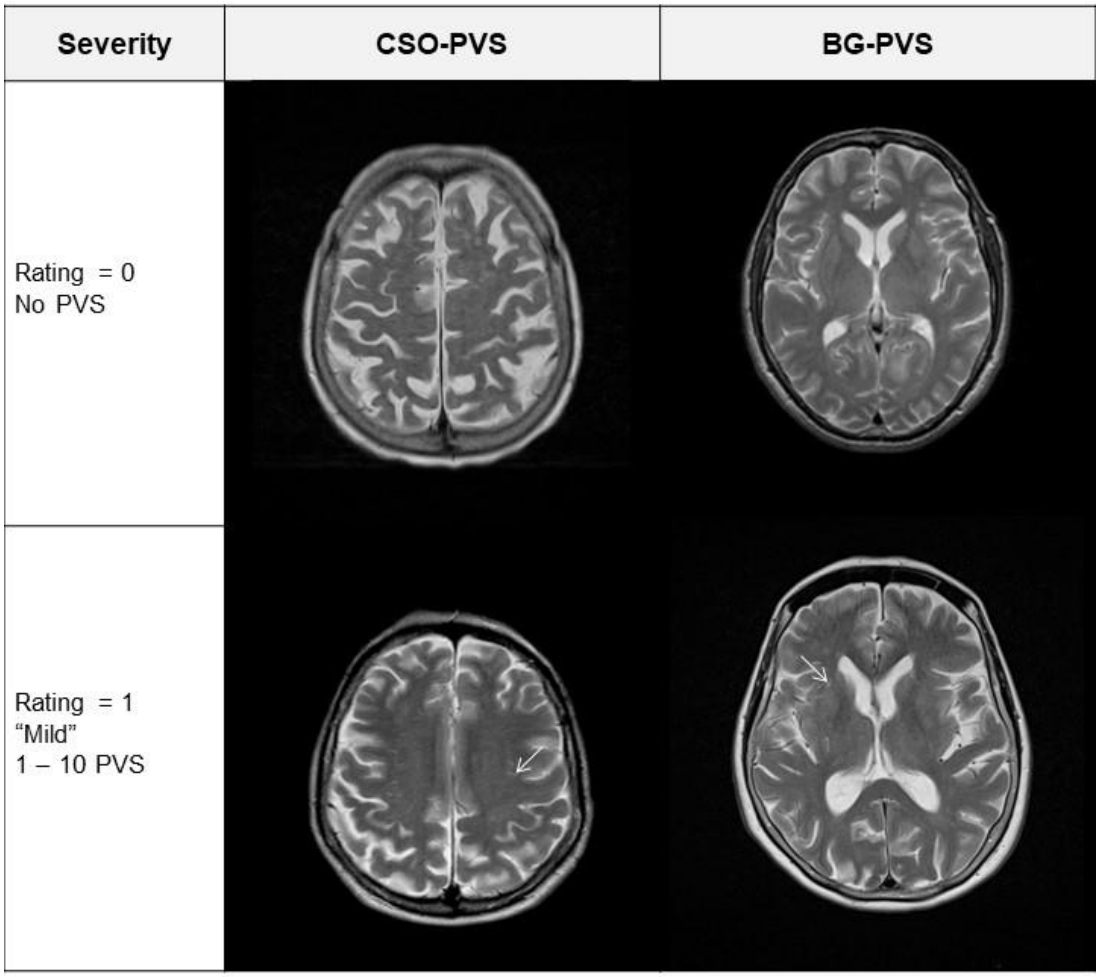
MRI-visible perivascular spaces (PVS) are defined by the STRIVE criteria as “fluid-filled spaces that follow the typical course of a vessel as it goes through grey or white matter” (7). They are CSF isointense, and usually appear as round or ovoid, but can appear as linear when imaged parallel to a vessel (rather than perpendicular to it) (7). They are distinguished from lacunes by the lack of a “halo” (hyperintense rim) on FLAIR imaging (7).

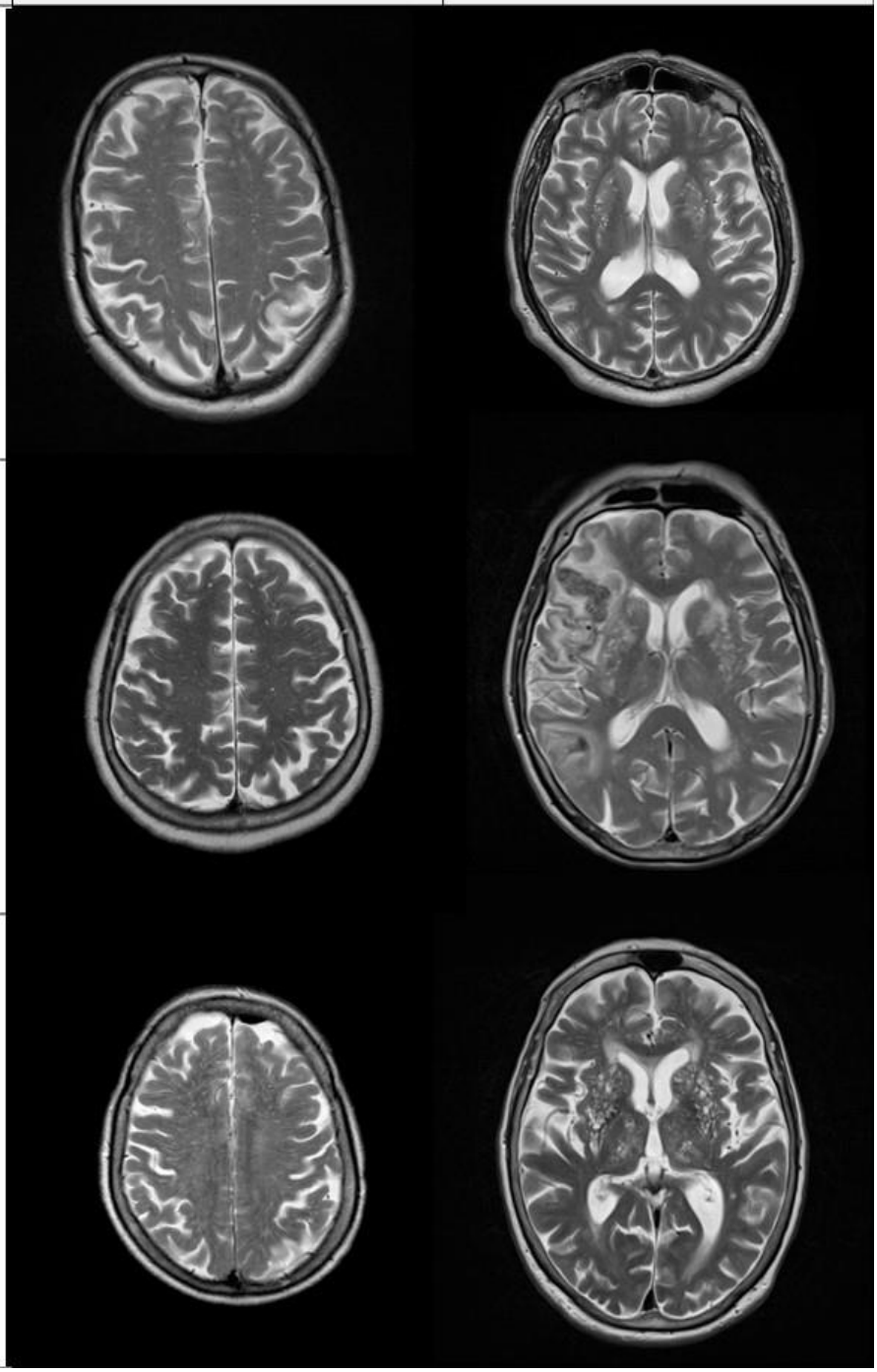
PVS were rated on axial T2-weighted MR images using a validated 4-point visual rating scale (0 = no PVS, 1 = <10 PVS, 2 = 11-20 PVS, 3 = 21-40 PVS and 4 = >40 PVS) in the basal ganglia (BG-PVS) and centrum semi-ovale (cerebral hemisphere white matter; CSO-PVS) (192, 193). Rating was carried out on a single pre-defined slice (first slice above the anterior commissure in the basal ganglia; the first slice above the level of the lateral ventricles for the centrum semi-ovale). Both hemispheres were counted, and the hemisphere with the highest score was recorded. The hemisphere contralateral to the acute stroke lesion (either ischaemic or haemorrhagic) was preferentially rated, and in cases where no lesion was present, the most severely affected side was included.

Examples of each grade of CSO-PVS and BG-PVS are shown in Figure 2.3.1.

Figure 2.3.1: Examples of PVS rating grades

Based on the scales described in (192, 193). Arrows indicate PVS, which are more apparent in the higher severity grades. The example shown for grade 3 BG-PVS also demonstrates evidence of gliotic change in the right hemisphere.



Severity	CSO-PVS	BG-PVS
Rating = 2 "Moderate" 11 – 20 PVS		
Rating = 3 "Frequent" 21 – 40 PVS		
Rating = 4 "Severe" >40 PVS		

2.3.2 White matter hyperintensities

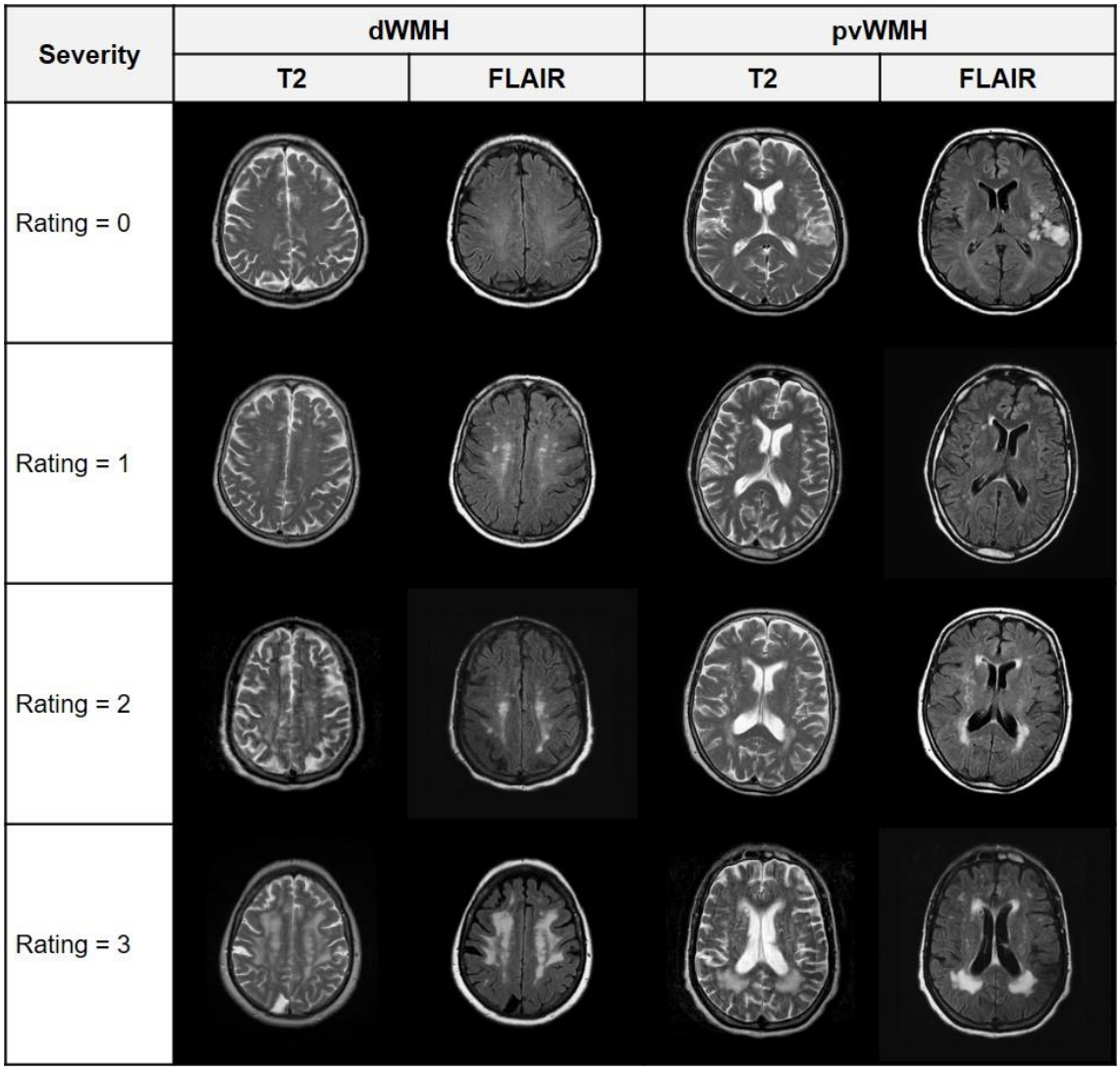
White matter hyperintensities (WMH; also termed leukoaraosis) were rated on T2 and FLAIR sequences using the Fazekas scale (194, 195). This scale considers WMH in deep (dWMH) and periventricular (pvWMH) distributions; each region is scored from 0 to 3 (Table 2.3.1). Examples of each rating grade are shown in Figure 2.3.2.

Table 2.3.1: The Fazekas scale for WMH

Adapted from (194).

Grade	dWMH	pvWMH
0	Absence	Absence
1	Punctate foci	“Caps” or “pencil-thin lining”
2	Early confluence of foci	Smooth “halo”
3	Large confluent areas	Irregular, extending into the deep white matter

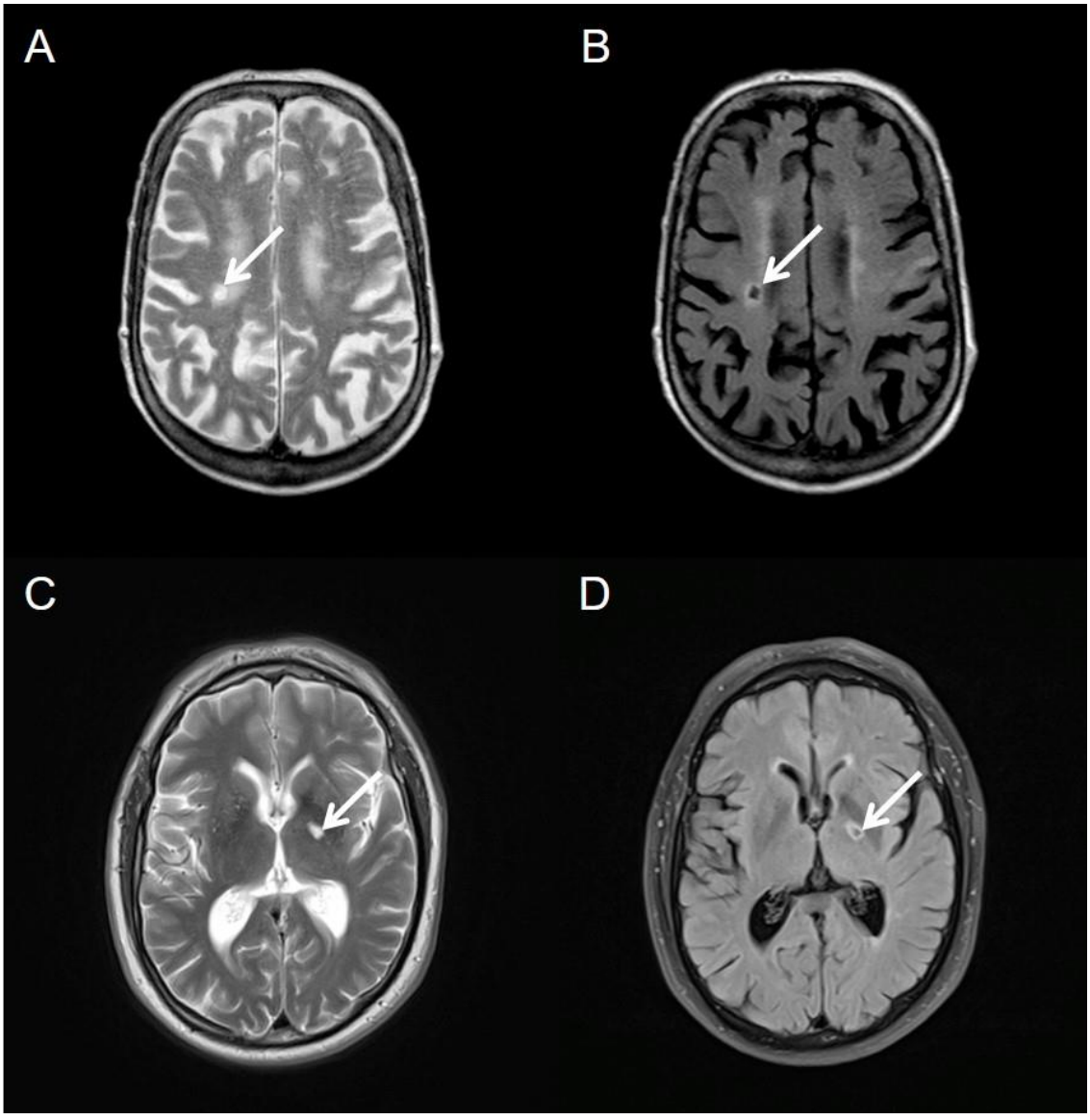
Figure 2.3.2: Rating WMH using the Fazekas scale
Examples of each rating grade on T2 and FLAIR imaging, for both deep (dWMH) and periventricular (pvWMH) regions. The example shown for pvWMH grade 0 also demonstrates previous damage (likely ischaemic) in the left hemisphere.



2.3.3 Lacunes

Lacunes were identified and counted on T2 and FLAIR sequences; they were defined as “round or ovoid, subcortical, fluid-filled (similar signal as CSF)” lesions, with a size between 3mm and 15mm and a “surrounding rim of hyperintensity” on FLAIR sequences, as defined in the STRIVE criteria (7). Examples are shown in Figure 2.3.3.

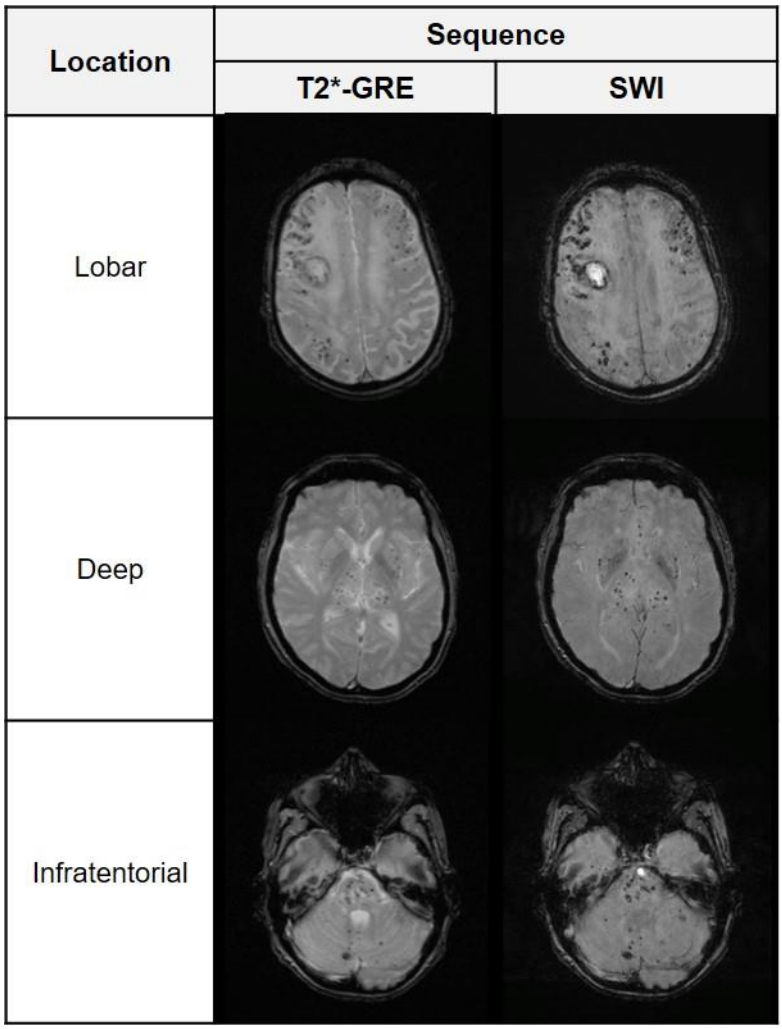
Figure 2.3.3: Lacunes
Axial T2-weighted (A, C) and FLAIR images (B, D) from two patients with lacunes (indicated by the arrows). In both cases, the T2 image shows a slightly irregular ovoid CSF isointense lesion, and the corresponding FLAIR image shows the characteristic hyperintense “halo”.



2.3.4 Cerebral microbleeds

Cerebral microbleeds (CMBs) were rated using blood sensitive sequences (either T2*-GRE or susceptibility weighted imaging, SWI) using the Microbleed Anatomical Rating Scale (MARS) (33).They were defined in accordance with STRIVE criteria (small, “generally 2–5 mm in diameter, up to 10 mm, areas of signal void with associated blooming”) (7). Examples of lobar, deep and infratentorial (cerebellar and brainstem) CMBs, using T2*-GRE and SWI (the same patient is shown for both sequences in a given location) are shown in Figure 2.3.4.

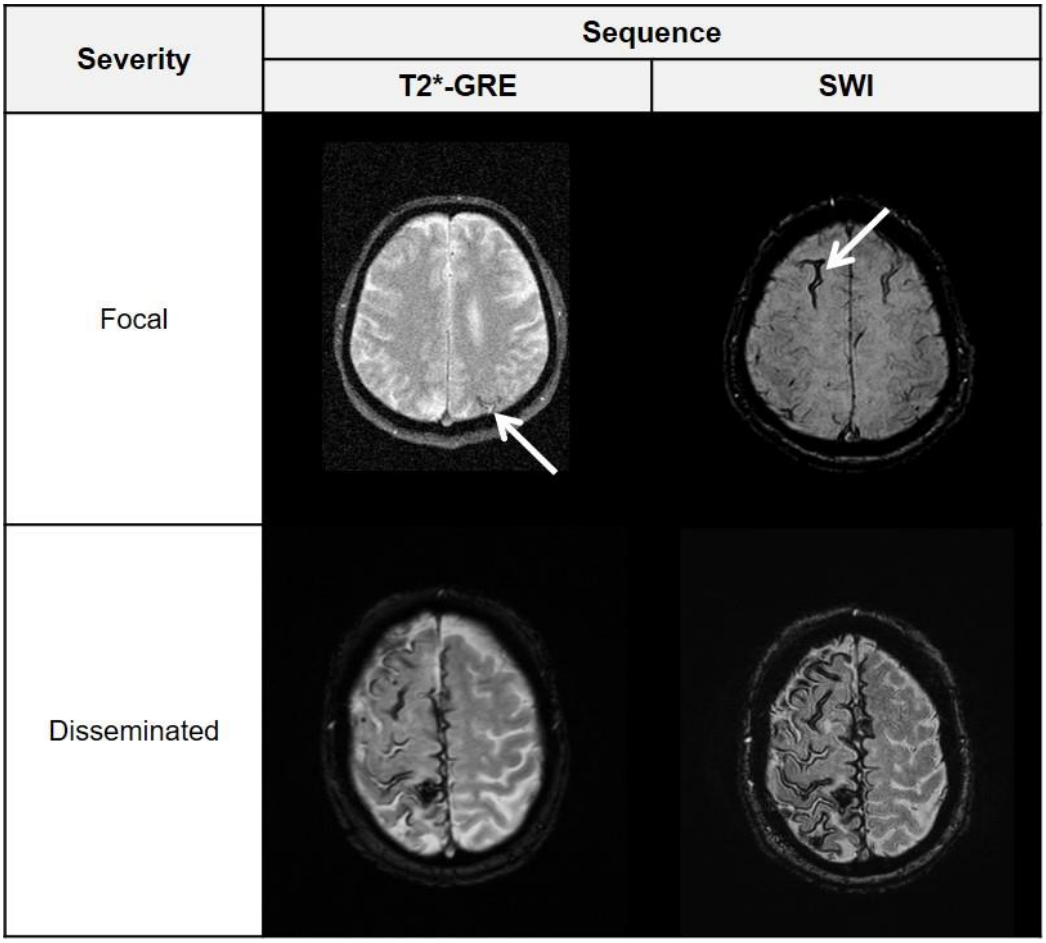
Figure 2.3.4: Examples of CMBs



2.3.5 Cortical superficial siderosis

Cortical superficial siderosis (cSS) is defined as curvilinear areas of low signal that follow the gyral cortical surface that can be identified on blood sensitive sequences (either T2*-GRE or SWI) (32). It is further classified as focal (involving three or fewer sulci) or disseminated (involving four or more sulci) (32). Examples are shown in Figure 2.3.5.

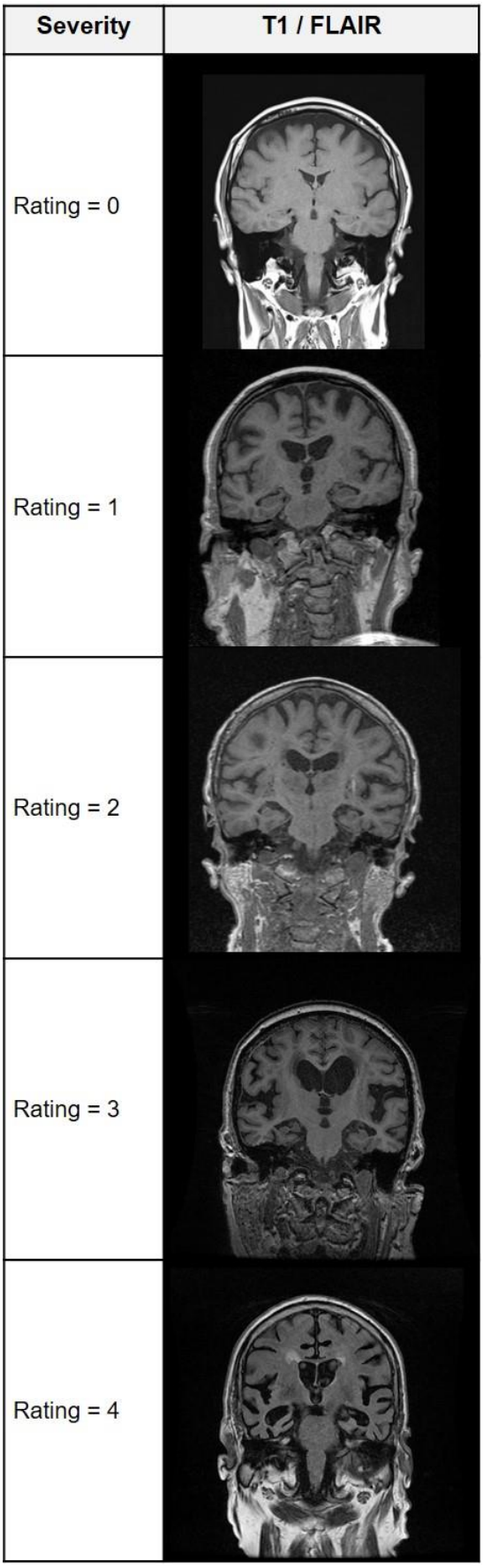
Figure 2.3.5: cSS
Examples of focal (two patients) and disseminated (one patient) cSS, using T2*-GRE and SWI sequences. Arrows indicate areas of cSS, which are more widespread in the disseminated case.



2.3.6 Medial temporal atrophy

Medial temporal atrophy (MTA) was rated on coronal T1 or FLAIR images using the Scheltens visual scale (196, 197). After review of the whole hippocampus, a slice in the middle of the hippocampal body was chosen for rating (196). The hemisphere contralateral to the acute stroke lesion (either ischaemic or haemorrhagic) was preferentially rated, and in cases where no lesion was present, the most severely affected side was included. There was good agreement between both sequences used (kappa 0.77). Examples of each severity grade are shown in Figure 2.3.6.

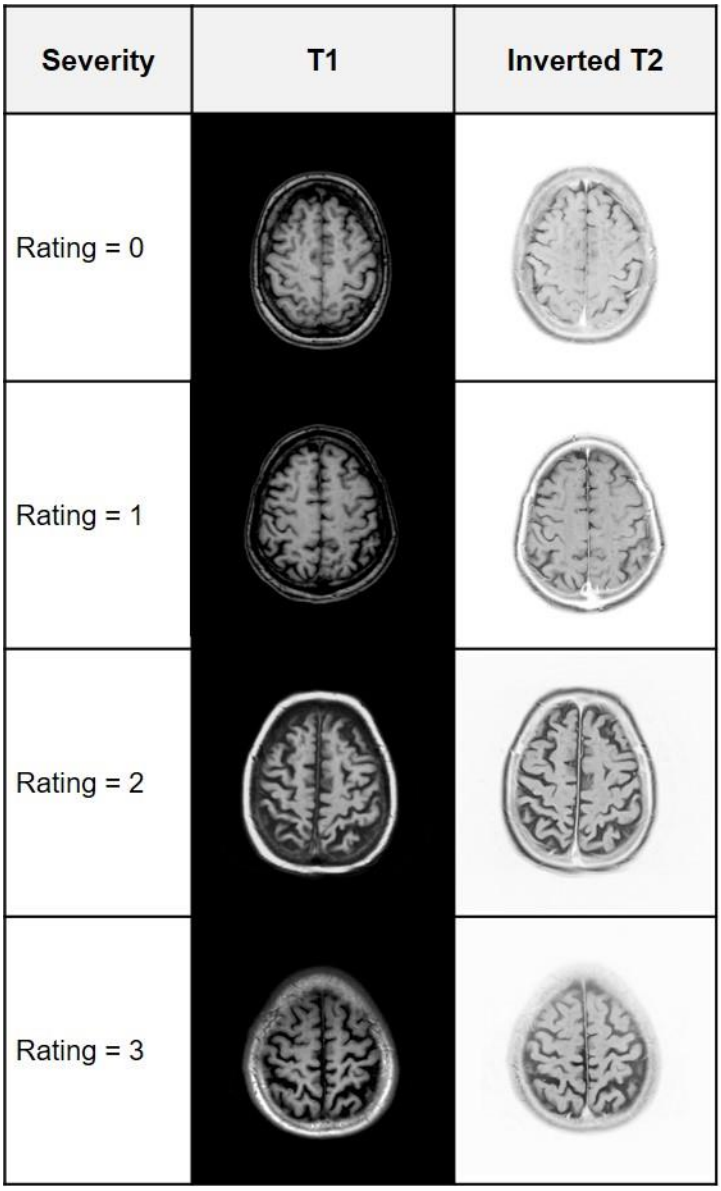
Figure 2.3.6: MTA rating using the Scheltens scale



2.3.7 Global cortical atrophy

Global cortical atrophy (GCA) was rated using the three-point Pasquier scale on axial T1 or FLAIR images; when these sequences were not available, inverted T2 images were used. The hemisphere contralateral to the acute stroke lesion (either ischaemic or haemorrhagic) was preferentially rated, and in cases where no lesion was present, the most severely affected side was included. There was good agreement between all sequences used (kappa 1.00). Examples of each severity grade are shown in Figure 2.3.7.

Figure 2.3.7: GCA



2.3.8 Composite CAA score

The “CAA score” was calculated from a previously described six-point scale (86). This scale awards 1 point for CSO-PVS rating of frequent to severe grades (i.e. presence of >20 CSO-PVS) and a further 1 point for significant WMH (defined as Fazekas pvWMH grade 3 or Fazekas grade dWMH grade ≥2, or both) (179). Additional points are awarded for the presence of lobar CMBs (1 point if 2 to 4 are present; 2 points if there are 5 or more) and cSS (1 point if focal; 2 points if disseminated) (86).

2.3.9 Composite SVD score

The “SVD score” was determined using a previously described four-point scale (179, 181). This scale awards 1 point each for the presence of lacunes, CMBs, moderate to severe BG-PVS (i.e. presence of >10 BG-PVS) and significant WMH (defined as Fazekas pvWMH grade 3 or Fazekas grade dWMH grade ≥2, or both) (179).

2.4 Topography of MRI-visible perivascular spaces in a memory clinic population

This section has been taken from published work (38). This project was undertaken in collaboration with Dr Sang Won Seo and colleagues at the Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea.

2.4.1 Introduction

SVDs and parenchymal Aβ deposition can both result in dementia, and are known to have a synergistic deleterious effect upon cognitive performance (198-200). Although these pathologies frequently coexist (201), they are associated clinically with different dementia syndromes: SVD is associated with fronto-executive dysfunction and subcortical vascular cognitive impairment (SVCI), whilst parenchymal Aβ deposition is correlated with episodic memory disturbances and AD (202, 203). AD and SVCI have been described as having distinct neuroimaging profiles (7, 8), but clinically differentiating between the two remains difficult, as both the cognitive symptoms and the imaging findings frequently overlap. Given this, identifying new markers that further improve our ability to discriminate between AD and SVCI remains both relevant and important, in particular with regard to recruitment for clinical trials investigating pharmacological interventions (204, 205).

As previously discussed, MRI-visible perivascular spaces (PVS) are hypothesised to result from an enlargement of the potential space within the wall of a cerebral blood vessel, possibly secondary to impaired interstitial fluid drainage (7, 37). BG-PVS appear to be associated with markers of DPA, whereas CSO-PVS are associated with cerebral Aβ pathologies (both AD and CAA) (40-42). Neuropathological studies have demonstrated that the frequency and severity of white matter PVS is greater in AD than controls, and this is associated with brain Aβ load, severity of CAA and Apolipoprotein E

(*APOE*) ε4 presence (206). The association between AD and increased white matter PVS volume has also been demonstrated using neuroimaging (207). CSO-PVS are associated with CAA-related ICH (42, 89) and its "haemorrhagic" markers, namely lobar CMBs (41, 208) and cSS (88). A study using post-mortem 7-Tesla MR in CAA-related ICH found an association between juxta-cortical PVS enlargement and the histopathological grade of CAA in the overlying cortex (209). There is also some evidence for an association between amyloid-PET burden (as measured using PiB-PET) and CSO-PVS (94).

This project aimed to establish the association of PVS location with SVD type in a cohort of patients with AD related cognitive impairment (ADCI; either AD or AD mild cognitive impairment) and SVCI (either subcortical vascular dementia or subcortical vascular mild cognitive impairment). We hypothesised that ADCI would be associated with CSO-PVS (as these patients are likely to have CAA), but not BG-PVS, which instead would be associated with SVCI (and DPA). We also hypothesised that, given the CSO-PVS are associated with cerebral Aβ diseases, CSO-PVS would be associated with PiB positivity, whereas BG-PVS would not demonstrate any such association.

2.4.2 Methods

This section is taken from published work by the candidate (162), and that by other authors (210); this cohort and the study methods have also been described in other published work by Dr Seo's group.

2.4.2.1 Participants

251 subjects with cognitive impairment were prospectively recruited between July 2007 and July 2011. All subjects were clinically diagnosed at the Samsung Medical Center, Seoul, Republic of Korea. In order to be included in the study, patients required a

diagnosis of subcortical vascular mild cognitive impairment (MCI), subcortical vascular dementia, probable Alzheimer's disease (AD) dementia or amnestic MCI.

Subcortical vascular MCI (n=67) was defined using a previously described modification of Petersen's criteria (211). Subcortical vascular dementia (n=70) was defined clinically using the Diagnostic and Statistical Manual of Mental Disorder Fourth Edition and using imaging criteria proposed by Erkinjuntti et al (212). Patients with subcortical MCI and subcortical vascular dementia all had severe white matter hyperintensities (WMH) on FLAIR, defined as periventricular WMH ≥ 10mm and deep WMH ≥ 25mm, as modified from the Fazekas ischaemia criteria (195).

Amnestic MCI (n=45) was defined by Petersen's criteria for mild cognitive impairment (MCI). Probable AD dementia (n=69) was defined using National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association criteria (213). Those with amnestic MCI or AD had WMH that were either minimal (periventricular WMH<5mm and deep WMH<5mm) or moderate (between minimal and severe WMH classifications).

Patients with territorial (i.e. large vessel) infarctions, WMH due to radiation injury, leukodystrophy, multiple sclerosis, or vasculitis were excluded. Whilst patients with large vessel infarctions were excluded, patients with a clinical history of lacunar stroke or deep intracerebral haemorrhage were not excluded. All patients underwent a clinical interview (for details including cardiovascular risk factors), neurological examination, cognitive assessment by a trained neuropsychologist, blood tests, *APOE* genotyping, PiB-PET and structural brain MRI.

This study was approved by Institutional Review Board of the Samsung Medical Center; written consent was obtained for each patient.

2.4.2.2 MRI acquisition

Standardized T2-weighted, three-dimensional (3D) T1-weighted turbo field echo, 3D FLAIR, T2* GRE and DTI sequences were acquired for all subjects at the Samsung Medical Center using the same 3-Tesla MRI scanner (Philips 3.0T Achieva). 3D T1-weighted turbo field echo MR images were acquired using the following parameters: sagittal slice thickness of 1.0 mm, over contiguous slices with 50% overlap; no gap; repetition time (TR) of 9.9 msec; echo time (TE) of 4.6 msec; flip angle of 8°; and matrix size of 240 × 240 pixels, reconstructed to 480 × 480 over a field of view (FOV) of 240 mm. The following parameters were used for the 3D FLAIR images: axial slice thickness of 2 mm; no gap; TR 11000 msec; TE 125 msec; flip angle 90°; and matrix size of 512 × 512 pixels. T2* GRE images were obtained using the following parameters: axial slice thickness of 5.0mm, inter-slice thickness of 2mm, TR 669 msec, TE 16 msec, flip angle 18°, and matrix size 560 × 560 pixels. In whole-brain DT-MRI examinations, sets of axial diffusion-weighted single-shot echo-planar images were collected using the following parameters: 128×128 acquisition matrix, 1.72 × 1.72 × 2 mm^3 voxels; 70 axial slices; 22 × 22 cm^2 FOV; TE 60 msec, TR 7696 msec; flip angle 90°; no gap; b-factor of 600 smm^{-2}. Diffusion-weighted images were acquired from 45 different directions using the baseline image without weighting [0, 0, 0]. All axial sections were acquired parallel to the anterior commissure-posterior commissure line.

2.4.2.3 PET acquisition and analysis

All patients completed a PiB-PET scan at either the Samsung Medical Center or the Asan Medical Center, using identical settings and a Discovery STe PET/CT scanner (GE Medical Systems, Milwaukee, WI, USA) in both cases. PiB-PET scanning was performed in 3D scanning mode that examined 35 slices of 4.25-mm thickness spanning the entire brain. PiB was injected into an antecubital vein as a bolus with a mean dose of 420 MBq (range 259 to 550 MBq). A CT scan was performed for attenuation correction 60 minutes

after injection. A 30-minute emission static PET scan was then initiated. The specific radioactivity of PiB at the time of administration was more than 1,500 Curie/mmol for patients and the radiochemical yield was more than 35%. The radiochemical purity of the tracer was more than 95% for all PET studies.

PiB-PET images were co-registered to individual MRIs, which were normalized to a Montreal Neurological Institute (MNI) 152 template (214). The quantitative regional values of PiB retention on the spatially normalized PiB images were obtained by an automated volume of interest (VOI) analysis using the automated anatomical labelling (AAL) atlas. Data processing was performed using SPM Version 5 (SPM5) within Matlab 6.5 (MathWorks, Natick, MA).

28 cortical VOIs from left and right hemispheres were selected using the AAL atlas. The cerebral cortical VOIs that were chosen for this study consisted of the bilateral frontal (superior and middle frontal gyri, the medial portion of superior frontal gyrus, the opercular portion of inferior frontal gyrus, the triangular portion of inferior frontal gyrus, supplementary motor area, orbital portion of the superior, middle, and inferior orbital frontal gyri, rectus and olfactory cortex), posterior cingulate gyri, parietal (superior and inferior parietal, supramarginal and angular gyri, and precuneus), lateral temporal (superior, middle and inferior temporal gyri, and heschl gyri), and occipital (superior, middle, and inferior occipital gyri, cuneus, calcarine fissure, and lingual and fusiform gyri). Regional cerebral cortical uptake ratios were calculated by dividing each cortical VOI's uptake ratio by the mean uptake of the cerebellar cortex (cerebellum crus1 and crus2), in order to obtain standardized uptake value ratios (SUVR). Global PiB uptake ratio was calculated from the volume-weighted average uptake ratio of bilateral 28 cerebral cortical VOIs. Patients were considered PiB-positive if their global PiB uptake ratio was greater than 1.5.

2.4.2.4 Structural markers of cerebral small vessel disease (SVD)

All rating was performed by trained individuals blinded to clinical details. PVS were rated by the candidate as described in the preceding Methods (Section 2.3.1). Severity was defined as “none/mild” (equivalent to rating scale categories 0 and 1), “moderate” (rating scale category 2), and “frequent/severe” (rating scale categories 3 and 4) in order to generate groups of a similar size for meaningful subsequent statistical analysis.

The remaining markers were rated by two experienced neurologists (Hee Jin Kim and Jae Seung Kim). WMH volume was measured on FLAIR images using an automated method (210). Lacunes and CMBs were rated as described in Section 2.3.3 and 2.3.4 respectively. Interrater agreement was 78.0% for lacunes and 92.3% for CMBs, and consensus was reached in all cases of discrepancy.

2.4.2.5 Statistics

Statistical analysis was performed by the candidate using Stata (Version 11.2). Baseline characteristics were compared using Chi-squared or Fishers exact tests for categorical variables, independent t-tests for normally distributed continuous variables and Mann Whitney U tests for continuous variables that were not normally distributed. PVS (both CSO-PVS and BG-PVS) were considered as categorical variables, subdivided by severity as described above. Univariable and multivariable logistic regression analyses were performed; variables of interest from the univariable analysis were included in the multivariable models. WMH volume and lacune burden were not included in the analysis for predictors of diagnosis, as these variables had been used to make the original clinical diagnosis.

2.4.3 Results

2.4.3.1 PVS topography as a predictor of clinical diagnosis

The baseline characteristics for the ADCI and SVCI groups are shown in Table 2.4.1. Those in the ADCI group were younger (mean age 70.3 years vs 73.8 years, p=0.0012), more likely to be PiB positive (78.2% vs 29.3%, p<0.0001) and carry the *APOE* ε4 allele (48.6% vs 25.7%, p<0.0001). Those in the SVCI group were more likely to have hypertension (77.6% vs 47.3%, p<0.0001), diabetes mellitus (25.9% vs 13.6%, p=0.021), hyperlipidemia (36.2% vs 23.6%, p=0.039), and prior stroke (26.7% vs 5.4%, p<0.0001). They were also more likely to carry the *APOE* ε3 allele (97.4% vs 86.9%, p=0.004) and have lacunes (median 9 vs 0, p<0.0001) and deep CMBs (53.5% vs 6.5%, p<0.0001).

In univariable logistic regression analysis (Table 2.4.2), increasing CSO-PVS severity was a positive predictor of ADCI; individuals with moderate CSO-PVS had an OR of 4.16 (95% CI, 2.08 to 8.29) and those with frequent/severe CSO-PVS had an OR of 9.43 (95% CI, 4.29 to 20.71) compared to those with none/mild CSO-PVS. Increasing severity of BG-PVS was negatively associated with ADCI (i.e. positively associated with a clinical diagnosis of SVCI); individuals with moderate BG-PVS had an OR for ADCI of 0.10 (95% CI, 0.04 to 0.26) and those with frequent/severe BG-PVS had an OR of 0.06 (95% CI, 0.01 to 0.47) compared to those with none/mild BG-PVS. After adjustment for other confounding variables, all of these associations remained: increasing CSO-PVS severity was a positive predictor of clinically diagnosed ADCI (none/mild as reference group: moderate severity, OR 3.57, 95% CI 1.17 to 10.89; frequent/severe, OR 6.26, 95% CI 1.66 to 23.58). Increasing severity of BG-PVS was negatively associated with ADCI and thus predictive of clinically diagnosed SVCI (none/mild as reference group: moderate severity, OR 0.26, 95% CI 0.07 to 1.01; frequent/severe, OR 0.03, 95% CI 0.00 to 0.44). PiB positivity and number of lacunes were also associated with a diagnosis of ADCI after adjustment.

2.4.3.2 PVS topography as a predictor of PiB positivity

The baseline characteristics of the PiB positive and negative groups are given in Table 2.4.3. Those in the PiB positive group were more likely to have a diagnosis of ADCI (71.7% vs 22.6%, p<0.0001), carry the *APOE* ε4 allele (53.9% vs 17.5%, p<0.0001) and have cSS, although the numbers were small (5.8% vs 0.9%, p=0.047). They were less likely to have hypertension (50.0% vs 77.4%, p<0.0001), diabetes mellitus (15.0% vs 25.5%, p=0.049), previous stroke (8.3% vs 25.5%, p=0.001) and the *APOE* ε3 allele (86.3% vs 99.0%, p<0.0001). PiB positive patients had lower WMH volumes (median 5.2ml vs 29.9ml, p<0.00001), fewer lacunes (median 0 vs 7, p<0.00001) and were less likely to have deep microbleeds (16.8% vs 46.7%, p<0.0001).

In univariable logistic regression analysis, increasing CSO-PVS severity was a positive predictor of PiB positivity; individuals with moderate CSO-PVS had an OR of 1.37 (95% CI, 0.74 to 2.54) and those with frequent/severe CSO-PVS had an OR of 2.50 (95% CI, 1.24 to 5.04) compared to those with none/mild CSO-PVS, respectively (Table 2.4.4). However, after adjustment for other factors, there was no relationship between CSO-PVS severity and PiB positivity. BG-PVS severity was not associated with PiB positivity. The only variables that remained independently associated with PiB positivity were ADCI diagnosis, presence of the *APOE* ε4 allele and number of lacunes.

Table 2.4.1: Baseline characteristics according to disease classification

p values reflect comparisons between ADCI and SVCI groups using Chi-squared, Fishers exact, independent t-tests or Mann Whitney U tests as appropriate.

	All	ADCI	SVCI	p value
n (%)	226	110 (48.7%)	116 (51.3%)	-
Age, years, mean (SD)	72.1 (8.1)	70.3 (8.8)	73.8 (7.0)	0.0012
Sex, male, n (%)	98 (43.4%)	49 (44.6%)	49 (42.3%)	0.727
Hypertension, n (%)	142 (62.8%)	52 (47.3%)	90 (77.6%)	<0.0001
Diabetes Mellitus, n (%)	45 (19.9%)	15 (13.6%)	30 (25.9%)	0.021
Hyperlipidemia, n (%)	68 (30.1%)	26 (23.6%)	42 (36.2%)	0.039
Prior stroke, n (%)	37 (16.4%)	6 (5.4%)	31 (26.7%)	<0.0001
Presence of *APOE* ε2, n (%)	22 (10.0%)	7 (6.5%)	15 (13.3%)	0.096
Presence of *APOE* ε3, n (%)	203 (92.3%)	93 (86.9%)	110 (97.4%)	0.004
Presence of *APOE* ε4, n (%)	81 (36.8%)	52 (48.6%)	29 (25.7%)	<0.0001
PiB Positivity, n (%)	120 (53.1%)	86 (78.2%)	34 (29.3%)	<0.0001
Lacunes, median (IQR)	1 (0 - 9)	0 (0 - 0)	9 (3.5 - 17)	<0.0001
cSS presence, n (%)	8 (3.5%)	5 (4.6%)	3 (2.6%)	0.426
Strictly lobar CMB (presence), n (%)	17 (7.5%)	7 (6.4%)	10 (8.6%)	0.520
Deep CMB (presence), n (%)	69 (30.8%)	7 (6.5%)	62 (53.5%)	<0.0001
CSO-PVS				
None/Mild (grade 0 - 1), n (%)	73 (32.3%)	16 (14.6%)	57 (49.1%)	<0.0001
Moderate (grade 2), n (%)	91 (40.3%)	49 (44.6%)	42 (36.2%)	
Frequent / Severe (grade 3 - 4), n (%)	62 (27.4%)	45 (40.9%)	17 (14.7%)	
BG-PVS				
None/Mild (grade 0 - 1), n (%)	170 (75.2%)	103 (93.6%)	67 (57.57%)	<0.0001
Moderate (grade 2), n (%)	44 (19.5%)	6 (5.4%)	38 (32.8%)	
Frequent / Severe (grade 3 - 4), n (%)	12 (5.3%)	1 (0.91%)	11 (9.48%)	

Table 2.4.2: Logistic regression analysis for predictors of clinical diagnosis (ADCI group)

	Univariable		Multivariable (CSO)		Multivariable (BG)	
	OR (95% CI)	**p value**	**OR (95% CI)**	**p value**	**OR (95% CI)**	**p value**
CSO-PVS: None/Mild (grade 0 - 1) Moderate (grade 2) Frequent / Severe (grade 3 - 4)	*Reference Group* 4.16 (2.08 to 8.29) 9.43 (4.29 to 20.71)	<0.00001	*Reference Group* 3.57 (1.17 to 10.89) 6.26 (1.66 to 23.58)	0.017	-	-
BG-PVS: None/Mild (grade 0 - 1) Moderate (grade 2) Frequent / Severe (grade 3 - 4)	*Reference Group* 0.10 (0.04 to 0.26) 0.06 (0.01 to 0.47)	<0.00001	-	-	*Reference Group* 0.26 (0.07 to 1.01) 0.03 (0.00 to 0.44)	0.009
Age (for each year older)	0.95 (0.91 to 0.98)	0.002	0.94 (0.88 to 1.01)	0.091	0.95 (0.88 to 1.03)	0.214
Hypertension (presence)	0.26 (0.15 to 0.46)	<0.0001	1.13 (0.39 to 3.28)	0.828	1.04 (0.35 to 3.11)	0.951
Diabetes (presence)	0.45 (0.23 to 0.90)	0.023	0.45 (0.14 to 1.44)	0.180	0.42 (0.13 to 1.36)	0.149
Hyperlipidaemia (presence)	0.55 (0.31 to 0.97)	0.041	0.39 (0.14 to 1.11)	0.077	0.53 (0.18 to 1.55)	0.247
Prior stroke (presence)	0.16 (0.06 to 0.40)	<0.0001	0.55 (0.12 to 2.56)	0.444	0.53 (0.12 to 2.28)	0.396
PiB positivity (presence)	8.64 (4.72 to 15.81)	<0.0001	3.97 (1.46 to 10.80)	0.007	5.63 (1.96 to 16.21)	0.001
APOE ε2 (presence)	0.46 (0.18 to 1.17)	0.103	-	-	-	-
APOE ε3 (presence)	0.18 (0.05 to 0.65)	0.009	0.47 (0.05 to 4.31)	0.507	0.29 (0.03 to 2.73)	0.277
APOE ε4 (presence)	2.73 (1.55 to 4.83)	0.001	0.82 (0.28 to 2.40)	0.722	0.65 (0.21 to 1.99)	0.449
Lacunes (per additional lacune)	0.49 (0.39 to 0.61)	<0.0001	0.61 (0.48 to 0.78)	<0.0001	0.59 (0.47 to 0.75)	<0.0001
cSS (presence)	1.79 (0.42 to 0.69)	0.432	-	-	-	-
Strictly lobar CMB (presence)	0.72 (0.26 to 1.96)	0.522	-	-	-	-
Deep CMB (presence)	0.06 (0.03 to 0.14)	<0.0001	0.45 (0.11 to 1.75)	0.247	0.59 (0.14 to 2.54)	0.478

Table 2.4.3: Baseline Characteristics for PiB positive and negative groups

P values reflect comparisons between PiB positive and negative groups using Chi-squared, Fishers exact, independent t-tests or Mann Whitney U tests as appropriate.

	PiB Negative (retention ratio < 1.5)	PiB Positive (retention ratio ≥ 1.5)	p value
n (%)	106 (46.9%)	120 (53.1%)	-
Age, years, mean (SD)	72.0 (7.2)	72.2 (8.8)	0.808
Sex, male, n (%)	48 (45.3%)	50 (41.7%)	0.584
Hypertension, n (%)	82 (77.4%)	60 (50.0%)	<0.0001
Diabetes Mellitus, n (%)	27 (25.5%)	18 (15.0%)	0.049
Hyperlipidemia, n (%)	37 (34.9%)	31 (25.8%)	0.138
Prior stroke, n (%)	27 (25.5%)	10 (8.3%)	0.001
ADCI, n (%)	24 (22.6%)	86 (71.7%)	<0.0001
SVCI, n (%)	82 (77.4%)	34 (28.3%)	<0.0001
Presence of *APOE* ε2, n (%)	14 (13.6%)	8 (6.8%)	0.096
Presence of *APOE* ε3, n (%)	102 (99.0%)	101 (86.3%)	<0.0001
Presence of *APOE* ε4, n (%)	18 (17.5%)	63 (53.9%)	<0.0001
WMH volume, ml, median (IQR)	29.9 (13.6 – 45.5)	5.2 (1.2 – 26.2)	<0.00001
Lacunes, median (IQR)	7 (1 – 17)	0 (0 – 2)	<0.00001
cSS presence, n (%)	1 (0.9%)	7 (5.8%)	0.047
Strictly lobar CMB (presence), n (%)	7 (6.6%)	10 (8.3%)	0.623
Deep CMB (presence), n (%)	49 (46.7%)	20 (16.8%)	<0.0001
CSO-PVS			
None/Mild (grade 0 - 1), n (%)	41 (38.7%)	32 (26.7%)	0.033
Moderate (grade 2), n (%)	44 (41.5%)	47 (39.2%)	
Frequent / Severe (grade 3 - 4), n (%)	21 (19.8%)	41 (34.2%)	
BG-PVS			
None/Mild (grade 0 - 1), n (%)	76 (71.7%)	94 (78.3%)	0.480
Moderate (grade 2), n (%)	23 (21.7%)	21 (17.5%)	
Frequent / Severe (grade 3 - 4), n (%)	7 (6.6%)	5 (4.2%)	

Table 2.4.4: Logistic regression analysis for predictors of PiB positivity

	Univariable		Multivariable	
	OR (95% CI)	p value	OR (95% CI)	p value
CSO-PVS: None/Mild (grade 0 - 1) Moderate (grade 2) Frequent / Severe (grade 3 - 4)	*Reference Group* 1.37 (0.74 to 2.54) 2.50 (1.24 to 5.04)	0.032	*Reference Group* 0.67 (0.29 to 1.59) 0.93 (0.35 to 2.46)	0.607
BG-PVS: None/Mild (grade 0 - 1) Moderate (grade 2) Frequent / Severe (grade 3 - 4)	*Reference Group* 0.74 (0.38 to 1.43) 0.58 (0.18 to 1.89)	0.480	-	-
Hypertension (presence)	0.29 (0.16 to 0.52)	<0.0001	0.52 (0.24 to 1.10)	0.085
Diabetes (presence)	0.52 (0.27 to 1.00)	0.051	0.97 (0.42 to 2.26)	0.947
Previous stroke (presence)	0.27 (0.12 to 0.58)	0.001	0.48 (0.17 to 1.33)	0.157
ADCI diagnosis	8.64 (4.72 to 15.81)	<0.0001	7.56 (2.59 to 22.46)	<0.0001
APOE ε2 (presence)	0.47 (0.19 to 1.16)	0.102		
APOE ε3 (presence)	0.06 (0.01 to 0.48)	0.007	0.25 (0.03 to 2.29)	0.221
APOE ε4 (presence)	5.51 (2.95 to 10.29)	<0.0001	3.87 (1.80 to 8.32)	0.001
WMH volume (for each ml higher)	0.97 (0.96 to 0.98)	<0.0001	1.03 (1.00 to 1.05)	0.055
Lacunes (for one number higher)	0.88 (0.83 to 0.92)	<0.0001	0.94 (0.88 to 0.99)	0.026
cSS (presence)	6.50 (0.79 to 53.76)	0.082	-	-
Strictly lobar CMB (presence)	1.29 (0.47 to 3.51)	0.623	-	-
Deep CMB (presence)	0.23 (0.12 to 0.43)	<0.0001	1.07 (0.43 to 2.65)	0.886

2.4.4 Discussion

CSO-PVS severity is strongly associated with clinically diagnosed ADCI whereas BG-PVS severity predicts clinically diagnosed SVCI. However, CSO-PVS severity was not independently associated with PiB positivity. There are two possible interpretations of this lack of independent association between CSO-PVS with PiB amyloid retention: either CSO-PVS are associated with ADCI as a marker of amyloid pathology that cannot be accurately resolved by amyloid-PET, or CSO-PVS are indicative of an amyloid-independent pathology.

Our findings are consistent with previous findings of an association between CSO-PVS and both AD and CAA, both of which are associated with Aβ deposition (14). One reason for the apparent lack of independent association between CSO-PVS and PiB might be that PiB-PET is unable to resolve smaller blood vessels affected by CAA. This is supported by neuropathological evidence that, although severity of CAA does appear associated with CSO-PVS in AD, the CAA affected vessels are predominantly less than 500μm in diameter, which may be too small to be identified using PiB-PET (206). Alternatively, the PiB-PET signal observed in our ADCI cohort may be more a measure of parenchymal Aβ (this being the predominant signal) and be unrepresentative of the true vascular Aβ; PiB-PET binding has been shown to be lower in patients with CAA compared to those with AD (215). Thus it is possible that any sequelae of vascular amyloid deposition, for example impaired interstitial fluid drainage secondary to a failure to adequately clear pathological proteins, could still be visible as MRI-visible CSO-PVS (37), independently of PiB positivity.

An alternative explanation is that CSO-PVS are associated with ADCI but not PiB positivity because they are manifestations of an amyloid-independent process, for example a tau protein related process. As well as being a core neuropathological finding in AD, neurofibrillary tangles have been demonstrated in association with CAA in patients

with AD (216), and tau deposits (neurofibrillary tangles and pretangles) have been described in Aβ-related angiitis, an inflammatory form of CAA (217). One study reviewing perivascular hyperphosphorylated tau in patients with AD found higher levels surrounding the CAA affected vessels than the unaffected ones (218). Thus it is possible that CAA could impair perivascular drainage, leading to tau aggregation, which could further impair perivascular drainage leading to further tau aggregation and so on (a "feed-forward" loop), with MRI-visible perivascular spaces being the end result (219, 220). In animal models, traumatic brain injury appears to disrupt normal perivascular clearance for at least 28 days, resulting in the accumulation of hyper-phosphorylated tau (221); CAA could impair perivascular drainage in a similar way. Alternatively CAA may disrupt perivascular drainage via perturbations in normal arteriolar pulsation (77, 222). It is also possible that the presence of hyper-phosphorylated tau has direct deleterious consequences for perivascular astrocytes, for example by directly disrupting their microtubular structure, or altering the expression or localisation of membrane channels (for example, aquaporin 4) that change normal interstitial fluid dynamics, with the eventual outcome of an enlarged perivascular space (223-225).

This project has some limitations. Firstly, this is an observational study without healthy aged matched controls for comparison; despite this, our findings are generally in keeping with previous reports from AD and SVCI cohorts. A previous study (94) demonstrated an association between PiB positivity and CSO-PVS across a cohort including healthy controls (both aged over and under 60 years) and patients with CAA-related ICH; interestingly although those with CAA had a higher burden of CSO-PVS compared with the healthy control groups ($p=0.08$), there did not appear to be a difference in PiB positivity between healthy older patients and CAA ($p=0.53$). This may provide further evidence that CSO-PVS burden is a closer correlate of vascular amyloid burden than PiB-PET measures are, but it is difficult to draw firm conclusions as this study included only 31 participants (94). Additionally, our findings may only be applicable to a selected

memory clinic population, specifically those with either ADCI or SVCI, rather than the full spectrum of dementia syndromes. Our project would also have strengthened if participants had other measures of Aβ burden in addition to amyloid-PET, for example quantification of CSF or serum Aβ. Certain measures, for example the ratio of Aβ-40:42 (150), may better capture vascular Aβ and thus might demonstrate with CSO-PVS. It was not possible to draw any conclusions on whether the association between AD diagnosis and CSO-PVS severity was due to any form of CAA. Only small numbers within our cohort had characteristics known to be associated with haemorrhage-associated CAA (also called type 2 CAA), namely an *APOE* ε2 allele (notably, none of the cohort were homozygous for *APOE* ε2), strictly lobar CMBs and cSS; however, given that over 95% of those with AD have pathological evidence of CAA it may be that the predominant CAA subtype in AD is type 1, which is associated with *APOE* ε4 and capillary level disease (14). Thus it may be the case that more traditional "haemorrhagic" markers of CAA are of less clinical relevance in this population.

This study provides further supporting evidence that CSO-PVS are a key imaging marker for AD, but without being a measure of amyloid positivity as measured by PiB-PET. This raises the possibility that CSO-PVS are a measure of vascular amyloid processes that are not identified by amyloid-PET (including those that might impair tracer uptake), or alternatively of an amyloid independent process, or both.

2.5 Cognitive impairment before intracerebral haemorrhage is associated with cerebral amyloid angiopathy

This section is taken from published work by the candidate (191), which was completed in collaboration with the listed authors.

2.5.1 Introduction

Although the associations between dementia and ischaemic stroke have been comprehensively described (226), fewer data are available for spontaneous ICH, in part due to its high case fatality (20, 123). Cognitive impairment often develops in survivors of ICH who were previously dementia-free, particularly if the ICH is lobar and associated with baseline neuroimaging markers of CAA (123). In those presenting with ICH, cognitive impairment before the event is common, with an estimated pooled incidence of 16.7% (133), suggesting that the underlying neurovascular and neuropathological processes that result in cognitive impairment following ICH might already be present at the time of initial presentation with ICH (123, 124, 133). However, it is not clear to what extent cognitive impairment after ICH is mediated by direct damage from the index ICH, the effects of recurrent ICH, or the impact of the underlying small vessel disease (123, 133); understanding the contribution of these mechanisms is potentially important in developing rational dementia prevention strategies.

We therefore investigated whether neuroimaging evidence of CAA (specifically, meeting the modified Boston criteria for probable CAA (57) at presentation, and increases in a composite CAA score (86)) was associated with the presence of cognitive impairment before ICH. We then performed further analyses investigating associations between individual MRI neuroimaging markers of small vessel disease and cognitive impairment before ICH.

2.5.2 Methods

2.5.2.1 Participants

We included patients recruited to a prospective multicentre observational cohort study of symptomatic patients with confirmed ICH. Those aged 18 years or above with an ICH confirmed on brain imaging (either CT or MRI) were eligible, providing there was no evidence that the ICH was due to an underlying structural cause or secondary to head trauma. This study has been preregistered (https://clinicaltrials.gov; NCT02513316) and the full details of the study protocol have been published previously (227). The study was approved by the National Research Ethics Service (IRAS reference 10/H0716/61). Written informed consent was obtained for each patient, either from the patient themselves (when they had capacity), or from a proxy (as defined by local regulations) in situations where the patient was unable to consent for themselves.

The Informant Questionnaire for Cognitive Decline in the Elderly (IQCODE) is a validated questionnaire given to a patient's family member or caregiver which aims to establish whether there have been specific changes in cognitive and functional performance over the preceding 10-year time period (228-230). Specifically, the informant was asked to compare the patient's performance from 10 years ago with their performance just before their stroke or TIA. The 16-item IQCODE includes 16 questions, each of which can be scored between 1 and 5; the total is then divided by 16, to provide the final score (range 1.0 to 5.0). This version of the IQCODE has been reported to have similar accuracy to the original 26-item version (229). Pre-existing cognitive impairment was defined as an IQCODE score>3.3; this threshold was based on data from a systematic review evaluating the diagnostic accuracy of the IQCODE for detecting clinically diagnosed dementia (of any cause) in secondary care environments (i.e. any hospital inpatient or outpatient setting, including emergency medical admissions and specialist cognitive

services) (229). An IQCODE threshold of 3.3 had the highest pooled sensitivity (0.96, 95% CI 0.94 to 0.98) of the thresholds investigated in the review; the sensitivity of this threshold was 0.66 (95% CI 0.41 to 0.84) (229).

For inclusion in the final analysis, it was necessary for patients to have an IQCODE from the time of their admission, together with the MRI sequences needed for imaging analysis (described below).

2.5.2.2 Imaging Acquisition and Analysis

Imaging was undertaken at each study centre according to local protocols, and all brain imaging carried out as part of the participant's standard clinical care were sent to the study's co-ordinating centre in anonymised DICOM format.

Imaging analysis was carried out by the candidate, in addition to another clinical research associate (Duncan Wilson) and two MSc students (Karen Osei-Bonsu Appiah, Surabhika Lunawat), all of whom were trained in neuroimaging rating and blinded to the participant clinical details. Only patients with an available MRI and all of the necessary sequences for cerebral small vessel disease rating (i.e. axial T2, axial and/or coronal FLAIR, and a blood sensitive sequence) were included in the neuroimaging analysis.

PVS, MTA and GCA were rated by the candidate; lacunes, CMBs and cSS were rated by Duncan Wilson, and WMH were rated by Karen Osei-Bonsu Appiah (in all cases using criteria described in Section 2.3). ICH location (rated by Duncan Wilson) was defined as either infratentorial, deep or lobar, with the latter in cortical or cortical-subcortical regions and not involving any of the deep grey matter structures. Haematoma volume was

calculated (Surabhika Lunawat) using a previously described validated semi-automated planimetric method (231).

A clinico-radiological diagnosis of “probable CAA” was based on meeting the modified Boston criteria (57). The CAA and SVD scores were calculated as described in Section 2.3.

2.5.2.3 Statistics

Statistical analysis was performed by the candidate using Stata (Version 11.2). Evidence of selection bias within the final cohort was evaluated by comparing the characteristics of people with appropriate MR imaging and those without (Table 2.5.1). IQCODE was dichotomised as described above, and baseline characteristics were compared for patients with scores >3.3 (i.e. with cognitive impairment) and those with scores ≤3.3 (Table 2.5.2). Continuous data were reviewed for normality, and if normally distributed we used the independent t-test. Where continuous variables were not normally distributed, we used the (non-parametric) Mann Whitney U test. We used the chi-squared tests for categorical variables. The independent t-test (normally distributed continuous data) and the two-sample test of proportion (categorical data) were used to compare means and proportions, respectively.

Univariate comparisons were used to identify potential confounders for inclusion in the multivariable models; all variables with $p<0.05$ were included. We then performed adjusted logistic regression analyses, adjusting for significant associations identified in univariate analyses (Table 2.5.2). In further analyses (Table 2.5.3) we investigated associations with other neuroimaging markers suggestive of CAA (the presence of strictly lobar CMBs, and presentation with lobar ICH), as well as a composite SVD score and its component elements. In these analyses, each neuroimaging marker was

considered individually (i.e. each adjusted model included only one neuroimaging marker at a time). Given that these analyses were exploratory, we did not make an adjustment for multiple testing.

2.5.3 Results

2.5.3.1 Cohort characteristics

The demographic and imaging characteristics of those included (n=166) are shown in Table 2.5.1. Patients without MRI (n=588) and those with MRI but with missing or uninterpretable sequences (n=43) were excluded (Figure 2.5.1). When compared to the excluded patients (Table 2.5.1), those included were younger (mean 68.9 years vs 75.0 years, p<0.00001), less likely to have hypertension (58.2% vs 70.9%, p=0.002), hypercholesterolaemia (35.8% vs 47.9%, p=0.006), diabetes mellitus (12.1% vs 19.8%, p=0.024), and AF (12.3% vs 43.5%, p<0.0001), and more likely to have a previous ischaemic stroke or TIA (24.7% vs 18.1%, p=0.081), lower GCS at presentation (IQR 13 to 15 vs 14 to 15, p=0.003) and IQCODE-defined pre-ICH cognitive decline (38.2% vs 24.7%, p=0.001).

When considering patients included in the study (Table 2.5.2), patients with IQCODE-defined pre-ICH cognitive impairment (n=41) were older (mean difference 7.5 years, p<0.0012), and more likely to have hypercholesterolaemia (51.2% vs 30.6%, p=0.017), diabetes mellitus (22.0% vs 8.9%, p=0.026), previous ischaemic stroke or TIA (29.0% vs 14.8%, p=0.047), and previous ICH (12.5% vs 3.2%, p=0.025).

2.5.3.2 Associations with pre-ICH cognitive decline: univariate and multivariate analyses

Univariate logistic regression analyses showed that pre-ICH cognitive decline was associated with meeting the modified Boston criteria for probable CAA at presentation and increasing CAA score (Table 2.5.3). In our multivariable analysis, we adjusted for age at event, hypercholesterolaemia, presence of diabetes mellitus, previous ischaemic stroke or TIA, and previous ICH, which were statistically significant in univariate analyses (Table 2.5.2). Meeting the modified Boston criteria for probable CAA at presentation (OR 4.01, 95% CI 1.53 to 10.51, p=0.005) and increasing CAA score (for each point increase, OR 1.42, 95% CI 1.03 to 1.97, p=0.033) remained associated with pre-ICH cognitive decline in these adjusted analyses (Table 2.5.3).

We then investigated the associations between individual neuroimaging markers of small vessel disease and cognitive impairment before ICH. In univariable analyses (Table 2.5.4), we identified associations between pre-ICH cognitive decline and increasing SVD score, WMH, the presence of cSS, presence of strictly lobar CMBs, and lobar ICH at presentation. In adjusted analyses (adjusted again for clinical and demographic variables identified in the univariate analysis) the presence of cSS (OR 4.08, 1.28 to 13.05, p=0.018), strictly lobar CMBs (OR 2.47, 95% CI 0.95 to 6.37, p=0.062) and lobar ICH at presentation (OR 2.29, 95% CI 0.99 to 5.31, p=0.053) showed associations with pre-ICH cognitive impairment. The previous associations with increasing SVD score and WMH were no longer statistically significant, although for WMH a large effect size remained (OR 2.03).

Figure 2.5.1: Description of the study population

Only those with an available MRI and the necessary sequences for cerebral small vessel disease rating (i.e. axial T2, axial and/or coronal FLAIR, and a blood sensitive sequence) were included in the neuroimaging marker analysis.

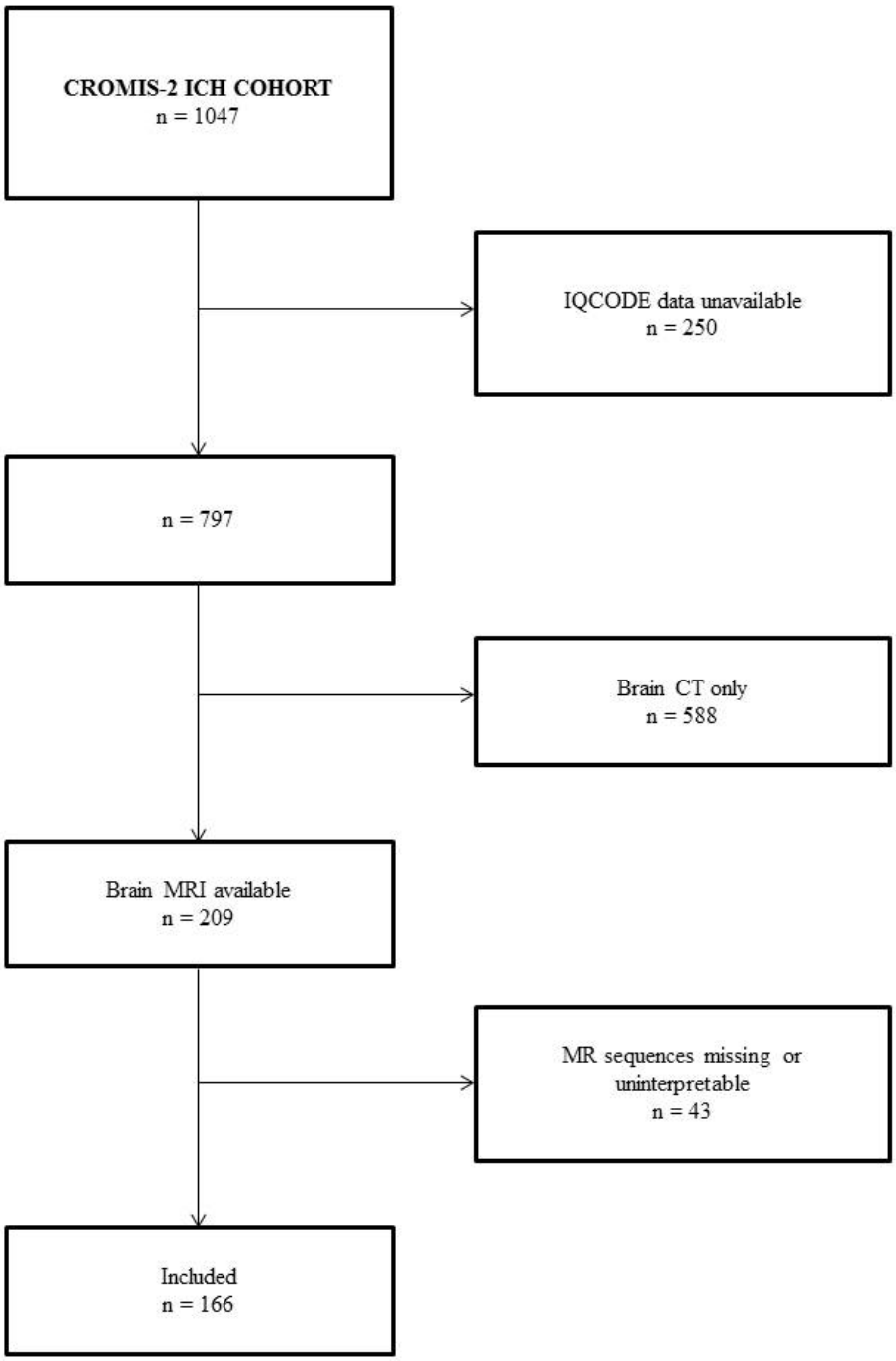


Table 2.5.1: Baseline characteristics of those included and excluded subjects

Percentage values were calculated using the total number of patients for whom data was available as the denominator. p values are from chi-squared and independent t-tests, except where indicated († for Mann Whitney U test).

	All with IQCODE	Included in final analysis	Excluded	p value
n	797	166	631	-
Age, years, mean (SD)	73.7 (12.1)	68.9 (12.9)	75.0 (11.6)	<0.00001
Sex, female, n (%)	328 (41.2)	62 (37.4)	266 (42.2)	0.263
Hypertension, presence, n (%)	539 (68.2)	96 (58.2)	443 (70.9)	0.002
Hypercholesterolaemia, presence, n (%)	351 (45.4)	58 (35.8)	293 (47.9)	0.006
Diabetes mellitus, presence, n (%)	144 (18.2)	20 (12.1)	124 (19.8)	0.024
AF, presence, n (%)	285 (38.8)	33 (21.3)	252 (43.5)	<0.0001
Previous ischaemic stroke or TIA, presence, n (%)	176 (23.3)	29 (18.1)	147 (24.7)	0.081
Previous intracerebral haemorrhage, presence, n (%)	38 (4.9)	9 (5.5)	29 (4.7)	0.683
GCS, median (IQR)	15 (14 – 15)	15 (14 – 15)	15 (13 – 15)	0.003†
IQCODE, median (IQR)	3.12 (3.0 – 3.5)	3.0 (3.0 – 3.3)	3.13 (3.0 – 3.5)	<0.00001†
IQCODE > 3.3	282 (35.4)	41 (24.7)	241 (38.2)	0.001

Table 2.5.2: Baseline demographic and clinical characteristics

Percentage values were calculated using the total number of patients for whom data was available as the denominator. p values are from chi-squared and independent t-tests. Proportion differences and their confidence intervals are given as percentages.

	All	IQCODE ≤3.3	IQCODE > 3.3	Mean or proportion difference (95% CI)	p value
n (%)	166	125 (75.3)	41 (24.7)	-	-
Age, years, mean (SD)	68.9 (12.9)	67.0 (13.1)	74.5 (10.9)	-7.5 (-11.9 to -3.0)	0.0012
Sex, male, n (%)	104 (62.7)	76 (60.8)	28 (68.3)	-7.5 (-24.1 to 9.1)	0.389
Hypertension, presence, n (%)	96 (58.1)	75 (60.5)	21 (51.2)	9.3 (-8.3 to 26.8)	0.297
Hypercholesterolaemia, presence, n (%)	58 (35.8)	37 (30.6)	21 (51.2)	-20.6 (-38.0 to -3.3)	0.017
Diabetes mellitus, presence, n (%)	20 (12.1)	11 (8.9)	9 (22.0)	-13.1 (-26.7 to 0.5)	0.026
AF, presence, n (%)	33 (21.3)	22 (19.0)	11 (28.2)	-9.2 (-25.1 to 6.6)	0.223
Previous ischaemic stroke or TIA, presence, n (%)	29 (18.1)	18 (14.8)	11 (29.0)	-14.2 (-29.9 to 1.5)	0.047
Previous ICH, presence, n (%)	9 (5.5)	4 (3.2)	5 (12.5)	-9.3 (-20.0 to 1.4)	0.025

Table 2.5.3: Univariable and adjusted logistic regression models, investigating associations between cognitive impairment before ICH and evidence of CAA

All adjusted models incorporated the following variables: age at event, hypercholesterolaemia, presence of diabetes mellitus, previous ischaemic stroke or TIA, and previous ICH.

	Univariable OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Meets modified Boston criteria for probable CAA	3.93 (1.72 to 8.96)	0.001	4.01 (1.53 to 10.51)	0.005
CAA score (per point increase)	1.45 (1.11 to 1.92)	0.007	1.42 (1.03 to 1.97)	0.033

Table 2.5.4: Logistic regression models (univariable and adjusted), reviewing associations between cognitive impairment before ICH and individual structural markers of cerebral SVD, and a composite SVD score.

Each model is independent, and considers only a single neuroimaging marker at a time.

All adjusted models incorporated the following variables: age at event, hypercholesterolaemia, presence of diabetes mellitus, previous ischaemic stroke or TIA, and previous ICH.

	Univariable OR (95% CI)	p value	Adjusted OR (95% CI)	p value
WMH; periventricular Fazekas 3 or deep Fazekas ≥2 (presence)	2.31 (1.11 to 4.79)	0.024	2.03 (0.87 to 4.74)	0.103
Lacunes, (presence)	1.18 (0.50 to 2.81)	0.702	-	-
CSO-PVS (per grade increase)	0.77 (0.53 to 1.12)	0.168	-	-
BG-PVS (per grade increase)	0.97 (0.53 to 1.80)	0.935	-	-
Strictly lobar CMBs (presence)	2.76 (1.21 to 6.30)	0.016	2.47 (0.95 to 6.37)	0.062
cSS (presence)	4.16 (1.55 to 11.12)	0.005	4.08 (1.28 to 13.05)	0.018
Presentation with lobar ICH	2.07 (1.00 to 4.28)	0.050	2.29 (0.99 to 5.31)	0.053
MTA (per grade increase)	1.33 (0.90 to 1.97)	0.150	-	-
GCA (per grade increase)	1.35 (0.88 to 2.08)	0.169	-	-
Haemorrhage volume (ml)	0.98 (0.96 to 1.01)	0.210	-	-
SVD score (per point increase)	1.52 (1.06 to 2.18)	0.021	1.36 (0.89 to 2.08)	0.150

2.5.4 Discussion

Our main new finding is that MRI neuroimaging markers of CAA are associated with pre-ICH cognitive impairment. This suggests that cognitive impairment in CAA is not only due to brain injury caused directly by ICH, but is also independently related to the underlying small vessel disruption associated with CAA.

Our findings add to growing evidence that CAA plays an important role in the development of cognitive impairment and dementia in those with ICH. The prevalence of pre-ICH dementia in lobar ICH is near double that in deep ICH (232), and structural imaging markers of CAA (cSS, CMBs) present at the time of ICH are associated with later progression to dementia (123). Our results show that a composite CAA score has a “per point” association with cognitive decline; further studies could help establish whether such a score might be useful in patients with milder CAA (including those not fulfilling Boston criteria, or without macrohaemorrhage). We found a strong association between cSS and pre-ICH cognitive impairment, suggesting that leptomeningeal haemorrhage, rather than parenchymal CMBs, might be an especially important pathological process impairing cognition in CAA. Our findings also contribute to our understanding of the mechanisms by which CAA disrupts cognition, which include haematoma damage (via direct effects on cortical integrity and function (123)) and small vessel mechanisms. The latter may include effects on brain network efficiency (102), which correlate with cognitive performance and shows disturbances in the non-ICH hemisphere (104). Our finding that CAA is associated with cognitive impairment before ICH shows that haematoma damage cannot be the only mechanism contributing to cognitive disruption, and supports the hypothesis that small vessel mechanisms are important.

A further possibility is that cognitive impairment prior to ICH is due to coincident Alzheimer's disease (133). Although the co-occurrence of CAA and Alzheimer's disease pathology is well recognised (51), CAA appears to have a cognitive profile distinct from that seen in Alzheimer's disease, characterised primarily by deficits in processing speed and executive function (125, 128). Recent neuropathological work (50) found that CAA makes an independent contribution to cognitive performance in Alzheimer's disease. Together, this evidence suggests that CAA has a specific "neurovascular" impact upon cognitive performance, independent of coexistent Alzheimer's pathology. Although we did not find an association between MTA or GCA (as putative imaging markers of Alzheimer's pathology (233)) and pre-ICH cognitive impairment, we acknowledge that our sample size is small and so we cannot rule out missing subtle effects.

The main strength of this study is our detailed neuroimaging description of the structural markers of cerebral small vessel disease in the context of pre-ICH cognitive decline, in a richly phenotyped prospective nationwide cohort of patients. However, our work also has limitations. Those included in our study were younger, with fewer comorbidities and lower IQCODE scores (and thus less IQCODE-defined cognitive impairment) than those who did not have an interpretable MRI; additionally, we acknowledge that a suspicion of CAA could increase the likelihood of an MRI being performed (50% of our included patients presented with lobar ICH), and so our final cohort might not be representative of those presenting with a spontaneous ICH to an acute stroke service. Brain imaging at each study centre was completed according to local protocols, and so there are unavoidable variations in the nature and manner of the sequences obtained, which could influence our results. In particular, the use of SWI versus T2*-weighted GRE sequences might result different CMB counts, as the former is more sensitive; we did not adjust for this in our analyses. There are inherent limitations of using the IQCODE, including variations in the thresholds used to define cognitive impairment and the lack of validation against a reference standard for pre-stroke cognitive impairment. Finally, we

acknowledge that our study size is small and so our results should be interpreted cautiously, particularly the adjusted analyses. As detailed, we chose not to apply an adjustment for multiple testing in order not to miss potential associations of interest. Whilst our study is powered to detect moderate effect sizes, it may have missed smaller effects.

Cognitive impairment before ICH is common, and associated with imaging findings consistent with an important contribution from CAA. This suggests that any future strategy aiming to reduce the impact of post-stroke dementia in ICH will need to extend beyond stroke prevention and include strategies that address the small vessel impact of CAA. Further work on the natural history of when and how CAA may influence an individual's cognitive profile is a priority for future research.

2.6 Cognitive impairment prior to atrial fibrillation related ischaemic events: neuroimaging and prognostic associations

This section was initially submitted for publication in August 2018 (Appendix 2, submitted manuscript II), and completed in collaboration with the authors listed.

2.6.1 Introduction

Post-stroke dementia is common, affecting up to 41.3% of patients in hospital populations (226). Atrial fibrillation (AF) is increasingly recognised as a key risk factor for dementia, both in association with and independently of clinically-overt ischaemic stroke, yet the mechanisms remain largely unknown (234-239). Possible causes include silent brain infarcts from recurrent embolization, cerebral hypoperfusion, chronic inflammation and endothelial dysfunction, or the progression of pre-existing cerebrovascular or neurodegenerative processes (240-253). It is likely that a proportion of post-stroke cognitive impairment is due to unidentified pre-stroke decline (226). The pooled prevalence of pre-stroke dementia is estimated to be 14.4% (in hospital-based cohorts, based on data from three studies), and is due to both neurodegenerative and vascular factors (226, 254, 255). Pre-stroke cognitive function is clinically relevant because it is associated with poor functional outcome, including death (256-260). However, most data on the clinical and radiological associations of pre-stroke cognitive impairment are from small single-centre studies in heterogeneous stroke populations which might not be generalisable to AF-related stroke populations (123, 133, 191, 260-270). Moreover, most imaging studies of pre-stroke cognitive impairment descriptions have focussed on global and regional atrophy measures and white matter changes, with limited descriptions of other important structural markers of small vessel disease (such as PVS and CMBs) which could provide new information on the underlying mechanisms.

We investigated the prevalence of IQCODE-defined pre-existing cognitive impairment in patients with ischaemic stroke or transient ischaemic attack (TIA) associated with AF, and its association with: (1) radiological markers of small vessel disease and neurodegeneration; (2) acute post-event cognitive performance as measured by the Montreal Cognitive Assessment (MoCA) (271, 272); and (3) functional outcome (defined by the modified Rankin score, mRS) at 24 months. We hypothesised that patients with IQCODE-defined cognitive impairment would have more evidence of small vessel disease and neurodegeneration that those without, and that pre-existing cognitive impairment would be associated with cognitive impairment in the acute period following the index event, as well as poorer functional outcomes at 24 months.

2.6.2 Methods

2.6.2.1 Patient selection

This is a pre-defined substudy nested within CROMIS-2 AF, a multi-centre prospective observational study of patients with cardioembolic stroke or TIA, the protocol for which has been described previously (227, 273). Briefly, this was a study of adults (aged 18 years or above) presenting with ischaemic stroke or TIA with non-valvular AF (confirmed by electrocardiography), who were eligible to start anticoagulation following their ischaemic event (227, 273). Patients who could not have an MRI scan, had contraindications to anticoagulation, or had previously received therapeutic anticoagulation, were excluded (227, 273). The study was approved by the National Research Ethics Service (IRAS reference 10/H0716/61).

Pre-existing cognitive impairment was identified using the 16-item IQCODE; details are described in Section 2.5.2.1. All patients with a baseline IQCODE were included in this analysis.

2.6.2.2 Imaging

Imaging was undertaken locally at each study centre in accordance with a standardised protocol including axial T2, T2*-GRE, diffusion-weighted imaging, coronal T1 and FLAIR images (227). Sequence parameters were specified for T2*-GRE (227); the remaining sequences were obtained according to local protocols. Imaging analysis was carried out by the candidate, and another clinical research associate (Duncan Wilson). Previous cortical infarcts were identified using T2 and FLAIR sequences (by the candidate), and confirmed as non-acute through comparison with diffusion-weighted images (DWI). Lacunes, PVS, MTA, and GCA were rated by the candidate; WMH, cSS and CMBs were rated by Duncan Wilson (in all cases using criteria described in Section 2.3). The presence of an acute DWI lesion was confirmed if a lesion was bright on the B1000 and dark on the corresponding ADC map (Duncan Wilson).

2.6.2.3 Outcome measures

Cognitive performance after the qualifying stroke event was measured using the MoCA, a scale including assessments of executive and attentional function sensitive to cognitive deficits secondary to cerebrovascular disease (271, 272, 274). This was requested for all participants at the time of recruitment, after the qualifying event. A MoCA score <26 was used to define cognitive impairment (274). The severity of cognitive impairment was defined as mild (MoCA score 18-25), moderate (MoCA score 10-17), and severe (MoCA score <10) based on guidance for scoring from the official website (http://www.mocatest.org/).

Functional outcome at 24 months was quantified using the modified Rankin scale (mRS) using multiple ascertainment methods to maximise follow up; these included postal questionnaires sent to patients and their general practitioners, and death notifications

from NHS Digital (previously the Health and Social Care Information Centre) (227, 275). The mRS was dichotomised at 2, with a score of ≤2 indicating independence (276).

2.6.2.4 Statistical analysis

We investigated for selection bias by comparing characteristics of those with and without a baseline IQCODE. We then compared baseline clinical, demographic and imaging findings in patients with and without pre-existing cognitive impairment. For all continuous variables, data were reviewed for normality, and if normally distributed the independent t-test was used. If variables were ordinal or not normally distributed, the non-parametric Mann Whitney U test was used. Chi-squared or Fisher's exact tests were used for categorical variables.

The results of univariable comparisons were used to identify variables for inclusion in multivariable logistic regression models; all variables with $p<0.20$ were included in the adjusted analyses, except for situations where variables both described the same phenomenon (for example, clinical history of previous ischaemic events and imaging evidence of a previous cortical infarct). The presence of one or more acute DWI lesions was used as a variable in all adjusted analyses for outcome, in order to control for the index event (i.e. stroke or TIA). Each model considered only a single neuroimaging marker at a time. Ordered logistic regression severity was used to analyse severity of acute MoCA impairment as an outcome; the regression was only adjusted for variables showing strong associations ($p<0.0001$) in univariable comparisons, as we did not want to over-adjust the model given the group sizes. The proportional odds assumption was investigated using the Brant test.

Post-hoc analyses were performed after excluding those with a pre-existing clinical diagnosis of dementia or cognitive impairment, previous ischaemic events or intracerebral haemorrhage at study entry. The reason for this was to establish whether

any findings in the cohort as a whole were driven by patients with these diagnoses, which are associated with cognitive impairment.

Statistical analyses were performed by the candidate using Stata (Version 15).

2.6.3 Results

Of the patients participating in the CROMIS-2 AF study (n=1490), we included 1102 patients for whom a baseline IQCODE was available; 388 patients were excluded as baseline IQCODE data was not available. The included patients were less likely to be current smokers (9.8% vs 16.9%, p<0.0001), and had a slightly higher educational age (mean 16.4 vs 16.9 years, p=0.027). There were other important but non-statistically significant differences between the two groups; the included patients more likely to have a formal diagnosis of dementia or cognitive impairment (2.8% vs 1.6%, p=0.166), less likely to have had a previous intracerebral haemorrhage (0.4% vs 1.1%, p=0.116), and more likely to be taking an antiplatelet drug at study entry (53.7% vs 48.7%, p=0.094).

2.6.3.1 Pre-existing cognitive impairment and associations

In our cohort, the mean IQCODE score was 3.2 (SD 0.6, score range 1.0 to 5.0), and 271 (24.6%) patients had IQCODE defined pre-existing cognitive impairment, of whom 23 (8.5%) had a known diagnosis of dementia or cognitive impairment at study entry. When comparing baseline clinical and demographic characteristics (Table 2.6.1), those with IQCODE-defined pre-existing cognitive impairment were older (mean age 79.2 vs 74.9 years, p<0.00001), and more likely to be female (49.1% vs 40.7%, p=0.015), have hypertension (70.0% vs 60.6%, p=0.009), diabetes mellitus (20.7% vs 15.7%, p=0.057), heart failure (6.6% vs 3.6%, p=0.034) a prior diagnosis of AF (37.7% vs 31.0%, p=0.042), a clinical history of previous ischaemic events (27.1% vs 16.5%, p<0.0001), be taking an

antiplatelet agent prior to their index event (62.1% vs 51.0%, p=0.002), and had a lower educational age (mean 15.7 vs 16.6 years, p=0.0003).

The neuroimaging features of the cohort are presented in Table 2.6.2. Those with pre-existing cognitive impairment were more likely to have previous cortical infarcts (24.1% vs 17.1%, p=0.011) and lacunes (22.1% vs 15.8%, p=0.020). They had higher grades of pvWMH (grade 3, 9.6% vs 3.6%, p<0.00001), dWMH (grade 3, 12.2% vs 4.5%, p<0.00001), BG-PVS (grade 2 or above, 28.8% vs 19.8%, p=0.0033), MTA (grades 3 and 4, 18.4% vs 5.8%, p<0.00001), and GCA (grades 2 and 3, 29.6% vs 22.7%, p=0.0078), and were more likely to have multiple CMBs (14.8% vs 8.5%, p=0.003). In multivariable logistic regression analysis (Table 2.6.3), in which each imaging predictor was considered separately, the presence of lacunes (OR 1.50, 95%CI 0.84 to 1.78, p=0.034), increasing pvWMH (per grade increase, OR 1.38, 95% CI 1.17 to 1.63, p<0.0001), dWMH (per grade increase, OR 1.26, 95% CI 1.05 to 1.51, p=0.011) and MTA (per grade increase, OR 1.61, 95% CI 1.34 to 1.95, p<0.0001) grade were independently associated with pre-existing cognitive impairment.

We then investigated whether IQCODE-defined cognitive impairment was associated with cognitive performance immediately after the entry ischaemic event (median time to assessment 4 days; n=960; Tables 2.6.4 and 2.6.5). Those with IQCODE-defined pre-existing cognitive impairment were more likely to have an abnormal MoCA score at study recruitment (84.2% vs 64.0% impaired, unadjusted OR 2.99, 95% CI 2.02 to 4.42, p<0.0001), which was maintained in adjusted analyses (OR 2.24, 95% CI 1.26 to 3.98, p=0.006). Pre-existing cognitive impairment was also associated with increasing severity of acute MoCA impairment in both unadjusted (per grade increase, OR 2.90, 95% CI 2.17 to 3.86, p<0.0001; Brant test p=0.519) and adjusted (OR 2.27, 95% CI 1.54 to 3.33, p<0.0001; Brant test p=0.753) analyses.

2.6.3.2 Outcome data

Outcome data at 24 months were available for 922 patients (83.7%) of whom 480 (52.1%) were functionally dependent (mRS > 2). Pre-existing cognitive impairment was associated with functional dependence at 24 months (n=157, 72.0%, vs n=323, 45.9%) in univariable (unadjusted OR 3.03, 95% CI 2.18 to 4.23, p<0.0001) and multivariable analyses (OR 2.43, 95% CI 1.42 to 4.20, p=0.001), adjusted for age at event, sex, hypertension, hypercholesterolaemia, diabetes mellitus, smoking, heart failure, clinical history of previous ischaemic events, educational age, admission NIHSS, antiplatelet use, pre-event mRS and the presence of an acute DWI lesion at study entry.

2.6.3.3 Subgroup analyses

We then repeated these analyses after excluding patients with a known clinical history of dementia, cognitive impairment, previous ischaemic events or intracerebral haemorrhage at study entry, in order to review whether the associations observed in the whole cohort were being driven by patients with these diagnoses. In patients without a known clinical history of dementia, cognitive impairment, previous ischaemic events or intracerebral haemorrhage at study entry, (n=872), the prevalence of IQCODE-defined pre-event cognitive impairment was 21.0% (n=183). The baseline clinical and demographic features were similar to those for the whole cohort (Table 2.6.6). The results of univariable and multivariable associations with pre-existing IQCODE were also consistent with our main findings (Tables 2.6.7, 2.6.8, 2.6.9, 2.6.10 and 2.6.11), except that the association with lacunes and pre-existing cognitive impairment no longer reached statistical significance in adjusted analyses.

Table 2.6.1: Baseline demographic and clinical characteristics

Comparison of baseline demographic and imaging characteristics between those with and without cognitive impairment prior to their qualifying event. Percentage values were calculated using the total number of patients for whom data was available as the denominator. p values are from independent t-tests (age at event, educational age), Mann Whitney U test (NIHSS), Fisher's exact test (previous intracerebral haemorrhage) or chi-squared tests (remainder).

	All	Pre-existing cognitive impairment		p value
		Absent	Present	
n (%)	1102	831 (75.4)	271 (24.6)	-
Age at event, years , mean (SD)	76.0 (10.1)	74.9 (10.1)	79.2 (9.4)	<0.00001
Sex, female, n (%)	471 (42.7)	338 (40.7)	133 (49.1)	0.015
Hypertension, n (%)	684 (62.8)	499 (60.6)	185 (70.0)	0.009
Hypercholesterolaemia, n (%)	496 (45.6)	370 (45.1)	126 (47.2)	0.555
Diabetes mellitus, n (%)	186 (16.9)	130 (15.7)	56 (20.7)	0.057
Smoking at study entry, n (%)	106 (9.8)	86 (10.5)	20 (7.6)	0.168
Heart failure, n (%)	48 (4.4)	30 (3.6)	18 (6.6)	0.034
Known AF, n (%)	356 (32.6)	255 (31.0)	101 (37.7)	0.042
Previous ischaemic event, n (%)	205 (19.1)	134 (16.5)	71 (27.1)	<0.0001
Previous intracerebral haemorrhage, n (%)	4 (0.4)	2 (0.2)	2 (0.8)	0.254
Educational age, years, mean (SD)	16.4 (3.5)	16.6 (3.8)	15.7 (2.4)	0.0003
Admission NIHSS, median (IQR)	5 (2 to 10)	5 (2 to 10)	4.5 (2 to 9)	0.9185
Antiplatelet use, n (%)	575 (53.7)	413 (51.0)	162 (62.1)	0.002

Table 2.6.2: Comparison of imaging features between those with and without pre-existing cognitive impairment

Percentage values were calculated using the total number of patients for whom data was available as the denominator. p values are from Mann Whitney U tests (pvWMH, dWMH, CSO-PVS, BG-PVS, MTA and GCA grades), Fisher's exact test (cSS) or chi-squared tests (remainder).

		All	Pre-existing cognitive impairment		p value
			Absent	Present	
n (%)		1102	831 (75.4)	271 (24.6)	-
Imaging evidence of previous cortical infarct, n (%)		207 (18.8)	142 (17.1)	65 (24.1)	0.011
Lacunes, presence, n (%)		188 (17.3)	130 (15.8)	58 (22.1)	0.020
pvWMH grade, n (%)	0	645 (58.5)	527 (63.4)	118 (43.5)	<0.00001
	1	206 (18.7)	149 (17.9)	57 (21.0)	
	2	195 (17.7)	125 (15.0)	70 (25.8)	
	3	56 (5.1)	30 (3.6)	26 (9.6)	
dWMH grade, n (%)	0	472 (42.8)	385 (46.3)	87 (32.1)	<0.00001
	1	431 (39.1)	315 (37.9)	116 (42.8)	
	2	129 (11.7)	94 (11.3)	35 (12.9)	
	3	70 (6.4)	37 (4.5)	33 (12.2)	
CSO-PVS grade, n (%)	0	58 (5.4)	44 (5.4)	14 (5.4)	0.5043
	1	486 (45.2)	361 (44.3)	125 (48.1)	
	2	324 (30.1)	255 (31.3)	69 (26.5)	
	3	174 (16.2)	128 (15.7)	46 (17.7)	
	4	33 (3.1)	27 (3.3)	6 (2.3)	
BG-PVS grade, n (%)	0	70 (6.4)	54 (6.6)	16 (6.0)	0.0033
	1	782 (71.6)	607 (73.7)	175 (65.3)	
	2	183 (16.8)	130 (15.8)	53 (19.8)	
	3	52 (4.8)	30 (3.6)	22 (8.2)	
	4	5 (0.5)	3 (0.4)	2 (0.8)	
MTA grade, n (%)	0	222 (22.0)	192 (24.9)	30 (12.6)	<0.00001
	1	470 (46.5)	373 (48.4)	97 (40.6)	
	2	229 (22.7)	161 (20.9)	68 (28.5)	
	3	66 (6.5)	38 (4.9)	28 (11.7)	
	4	23 (2.3)	7 (0.9)	16 (6.7)	
GCA grade, n (%)	0	355 (32.6)	282 (34.3)	73 (27.3)	0.0078
	1	469 (43.1)	354 (43.1)	115 (43.1)	
	2	246 (22.6)	174 (21.2)	72 (27.0)	
	3	19 (1.7)	12 (1.5)	7 (2.6)	
cSS, presence, n (%)		3 (0.3)	1 (0.1)	2 (0.7)	0.151
CMB, presence, n (%)		230 (20.9)	165 (19.9)	65 (24.0)	0.146
Presence of >1 CMB, n (%)		111 (10.1)	71 (8.5)	40 (14.8)	0.003

Table 2.6.3: Multivariable logistic regression for imaging predictors of pre-existing cognitive impairment

Each model considered only a single neuroimaging marker at a time.
†Adjusted for age at event, sex, hypertension, diabetes mellitus, smoking, heart failure, known AF, educational age, and antiplatelet use.
All remaining models were adjusted for age, sex, hypertension, diabetes mellitus, smoking, heart failure, clinical history of previous ischaemic events, known AF, educational age, and antiplatelet use.

	OR	95% CI	p value
Imaging evidence of previous cortical infarct, presence†	1.23	0.84 to 1.78	0.288
Lacunes, presence†	1.50	1.03 to 1.05	0.034
pvWMH, per grade increase	1.38	1.17 to 1.63	<0.0001
dWMH, per grade increase	1.26	1.05 to 1.51	0.011
BG-PVS, per grade increase	1.16	0.92 to 1.47	0.212
MTA, per grade increase	1.61	1.34 to 1.95	<0.0001
GCA, per grade increase	1.06	0.86 to1.31	0.588
cSS, presence	8.21	0.72 to 94.5	0.091
CMB, presence	1.10	0.76 to 1.58	0.620
Presence of >1 CMB	1.49	0.93 to 2.38	0.093

Table 2.6.4: Comparison of acute cognitive performance in those with and without IQCODE-defined pre-existing cognitive impairment

p values are derived from Mann Whitney U tests (days between index event and date of MoCA assessment) or chi squared tests (remainder).

	All	Pre-existing cognitive impairment		p value
		Absent	Present	
n (%)	960	739 (77.0)	221 (23.0)	-
Days between index event and date of MoCA assessment, median (IQR)	4 (2 to 9)	3 (2 to 8)	4 (2 to 10)	0.0631
Presence of acute cognitive impairment (MoCA score <26), n (%)	659 (68.7)	473 (64.0)	186 (84.2)	<0.0001
Degree of acute cognitive impairment, n (%)				
Normal, score ≥26	301 (31.4)	266 (36.0)	35 (15.8)	<0.0001
Mild deficit, score 18 - 25	449 (46.8)	344 (46.6)	105 (47.5)	
Moderate deficit, score 10 - 17	151 (15.7)	99 (13.4)	52 (23.5)	
Severe deficit, score <10	59 (6.2)	30 (4.1)	29 (13.1)	

Table 2.6.5: Logistic regression models reviewing associations between IQCODE-defined pre-existing cognitive impairment and acute cognitive performance

MoCA impaired models were adjusted for age at event, sex, hypertension, hypercholesterolaemia, diabetes mellitus, heart failure, history of known AF, educational age, admission NIHSS, antiplatelet use prior to study entry, and presence of an acute DWI lesion.
Severity of MoCA impairment models used ordinal logistic regression, and were adjusted for age, hypertension, educational age, NIHSS, and presence of an acute DWI lesion.

	Univariable OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Acute MOCA impaired (score <26)	2.99 (2.02 to 4.42)	<0.0001	2.24 (1.26 to 3.98)	0.006
Severity of acute MoCA impairment (normal, mild, moderate, severe), per increase in grade	2.90 (2.17 to 3.86)	<0.0001	2.27 (1.54 to 3.33)	<0.0001

Table 2.6.6: Baseline demographic and clinical characteristics, excluding those with a clinical diagnosis of dementia or cognitive impairment, previous ischaemic events or intracerebral haemorrhage at study entry

Comparison of baseline demographic and imaging characteristics between those with and without cognitive impairment prior to their qualifying event. Percentage values were calculated using the total number of patients for whom data was available as the denominator. p values are from independent t-tests (age, educational age), Mann Whitney U test (NIHSS), Fisher's exact test (previous intracerebral haemorrhage) or chi-squared tests (remainder).

	All	Pre-existing cognitive impairment		p value
		Absent	Present	
n (%)	872	689 (79.0)	183 (21.0)	-
Age at event, years , mean (SD)	75.1 (10.2)	74.2 (10.2)	78.5 (9.7)	<0.00001
Sex, female, n (%)	368 (42.2)	280 (40.6)	88 (40.1)	0.070
Hypertension, n (%)	519 (60.1)	400 (58.6)	119 (66.1)	0.066
Hypercholesterolaemia, n (%)	361 (41.9)	286 (42.0)	75 (41.7)	0.936
Diabetes mellitus, n (%)	137 (15.8)	102 (14.9)	35 (19.1)	0.158
Smoking at study entry, n (%)	91 (10.6)	75 (11.0)	16 (8.9)	0.406
Heart failure, n (%)	33 (3.8)	21 (3.1)	12 (6.6)	0.027
Known AF, n (%)	271 (31.4)	206 (30.2)	65 (35.9)	0.138
Educational age, years, mean (SD)	16.5 (3.7)	46.7 (3.2)	15.7 (2.4)	0.0031
Admission NIHSS, median (IQR)	5 (2 to 10)	5 (2 to 10)	5 (2 to 10)	0.9840
Antiplatelet use, n (%)	395 (46.8)	300 (44.9)	95 (53.7)	0.038

Table 2.6.7: Comparison of imaging features between those and without pre-existing cognitive impairment, excluding those with a clinical diagnosis of dementia or cognitive impairment, previous ischaemic events or intracerebral haemorrhage at study entry

Percentage values were calculated using the total number of patients for whom data was available as the denominator. p values are from Mann Whitney U tests (pvWMH, dWMH, CSO-PVS, BG-PVS, MTA and GCA grades), Fisher's exact test (cSS) or chi-squared tests (remainder).

		All	Pre-existing cognitive impairment		p value
			Absent	Present	
n (%)		872	689 (79.0)	183 (21.0)	-
Imaging evidence of previous cortical infarct, n (%)		130 (14.9)	93 (13.5)	37 (20.3)	0.021
Lacunes, presence, n (%)		132 (15.4)	97 (4.2)	35 (19.7)	0.073
pvWMH grade, n (%)	0	531 (60.9)	446 (64.7)	85 (46.5)	<0.00001
	1	166 (19.0)	124 (18.0)	42 (23.0)	
	2	141 (16.2)	97 (14.1)	44 (24.0)	
	3	34 (3.9)	22 (3.2)	12 (6.6)	
dWMH grade, n (%)	0	396 (45.4)	337 (48.9)	59 (32.2)	<0.00001
	1	337 (38.7)	253 (36.7)	84 (45.9)	
	2	95 (10.9)	72 (10.5)	23 (12.6)	
	3	44 (5.1)	27 (3.9)	17 (9.3)	
CSO-PVS grade, n (%)	0	50 (5.9)	38 (5.6)	12 (6.9)	0.9310
	1	375 (44.0)	298 (44.0)	77 (44.0)	
	2	261 (30.6)	212 (31.3)	49 (28.0)	
	3	142 (16.7)	111 (16.4)	31 (17.7)	
	4	24 (2.8)	18 (2.7)	6 (3.4)	
BG-PVS grade, n (%)	0	61 (7.1)	47 (6.9)	14 (7.8)	0.0422
	1	624 (72.1)	508 (74.2)	116 (64.4)	
	2	141 (16.3)	104 (15.2)	37 (20.6)	
	3	36 (4.2)	23 (3.4)	13 (7.2)	
	4	3 (0.4)	3 (0.4)	0 (0.0)	
MTA grade, n (%)	0	193 (24.3)	169 (26.6)	24 (15.1)	<0.00001
	1	375 (47.2)	311 (49.0)	64 (40.3)	
	2	162 (20.4)	120 (18.9)	42 (26.4)	
	3	50 (6.3)	31 (4.9)	19 (12.0)	
	4	14 (1.8)	4 (0.6)	10 (6.3)	
GCA grade, n (%)	0	285 (33.1)	236 (34.7)	49 (27.2)	0.106
	1	378 (43.9)	300 (44.1)	78 (43.3)	
	2	184 (21.4)	137 (20.1)	47 (26.1)	
	3	14 (1.6)	8 (1.2)	6 (3.3)	
cSS, presence, n (%)		1 (0.1)	1 (0.2)	0 (0.0)	1.000
CMB, presence, n (%)		173 (19.8)	133 (19.3)	40 (21.9)	0.441
Presence of >1 CMB, n (%)		77 (8.8)	55 (8.0)	22 (12.0)	0.087

Table 2.6.8: Multivariable logistic regression for imaging predictors of pre-existing cognitive impairment, excluding those with a clinical diagnosis of dementia or cognitive impairment, previous ischaemic events or intracerebral haemorrhage at study entry

Each model considered only a single neuroimaging marker at a time. Models adjusted for age, sex, hypertension, diabetes mellitus, heart failure, known AF, educational age, and antiplatelet use.

	OR	95% CI	p value
Imaging evidence of previous cortical infarct, presence	1.27	0.79 to 2.02	0.326
Lacunes, presence	1.47	0.94 to 2.31	0.093
pvWMH, per grade increase	1.32	1.08 to 1.61	0.006
dWMH, per grade increase	1.29	1.05 to 1.60	0.016
BG-PVS, per grade increase	1.03	0.77 to 1.36	0.854
MTA, per grade increase	1.55	1.25 to 1.94	<0.0001
GCA, per grade increase	1.09	0.85 to 1.39	0.503
CMB, presence	0.90	0.57 to 1.40	0.629
Presence of >1 CMB	1.13	0.63 to 2.05	0.679

Table 2.6.9: Comparison of acute cognitive performance in those with and without IQCODE-defined pre-existing cognitive impairment, excluding those with a clinical diagnosis of dementia or cognitive impairment, previous ischaemic events or intracerebral haemorrhage

p values are derived from Mann Whitney U tests (days between index event and date of MoCA assessment) or chi squared tests (remainder).

	All	Pre-existing cognitive impairment		p value
		Absent	Present	
n (%)	766	615 (80.3)	151 (19.7)	-
Days between index event and date of MoCA assessment, median (IQR)	4 (2 to 9)	4 (2 to 8)	4 (2 to 9)	0.6842
Presence of acute cognitive impairment (MoCA score <26), n (%)	516 (67.4)	390 (63.4)	126 (83.4)	<0.0001
Degree of acute cognitive impairment, n (%)				
Normal, score ≥26	250 (32.6)	225 (36.6)	25 (16.6)	<0.0001
Mild deficit, score 18 - 25	359 (46.9)	284 (46.2)	75 (49.7)	
Moderate deficit, score 10 - 17	114 (14.9)	80 (13.0)	34 (22.5)	
Severe deficit, score <10	43 (5.6)	26 (4.2)	17 (11.3)	

Table 2.6.10: Logistic regression models reviewing associations between IQCODE-defined pre-existing cognitive impairment and acute cognitive performance, excluding those with a clinical diagnosis of dementia or cognitive impairment, previous ischaemic events or ICH

MoCA impaired models were adjusted for age at event, sex, hypertension, hypercholesterolaemia, diabetes mellitus, heart failure, history of known AF, educational age, admission NIHSS, antiplatelet use prior to study entry, and presence of an acute DWI lesion.
Severity of MoCA impairment models used ordinal logistic regression, and were adjusted for age, hypertension, educational age, NIHSS, and presence of an acute DWI lesion.

	Univariable OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Acute MOCA impaired (score <26)	2.91 (1.84 to 4.60)	<0.0001	2.60 (1.28 to 5.28)	0.008
Severity of acute MoCA impairment (normal, mild, moderate, severe), per increase in grade	2.66 (1.89 to 3.72)	<0.0001	2.35 (1.49 to 3.70)	<0.0001
	Brant test	0.704	Brant test	0.261

Table 2.6.11: Logistic regression models reviewing associations between IQCODE-defined pre-existing cognitive impairment and functional outcome at 24 months

Multivariable model adjusted for age at event, sex, hypertension, hypercholesterolaemia, diabetes mellitus, smoking, heart failure, clinical history of previous ischaemic events, educational age, admission NIHSS, antiplatelet use, pre-event mRS and the presence of an acute DWI lesion at study entry.

	Univariable OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Functional dependence (mRS > 2)	2.78 (1.88 to 4.10)	<0.0001	3.33 (1.72 to 6.42)	<0.0001

2.6.4 Discussion

In our large multi-centre prospective cohort of patients with AF-associated ischaemic stroke and TIA, we found that nearly a quarter of patients (24.6%) met IQCODE criteria for pre-existing cognitive impairment, which was associated with the presence of lacunes, periventricular and deep WMH, and medial temporal atrophy, but not with other structural markers of small vessel disease (MRI-visible perivascular spaces, cortical superficial siderosis or cerebral microbleeds). We found that IQCODE-defined cognitive impairment was associated with both acute post-event cognitive performance and functional outcome at 24 months.

Our findings in an AF-associated cohort are in keeping with previous studies that have shown that pre-existing cognitive impairment is associated with both neurodegenerative and vascular factors (260-270). We found rates of pre-existing cognitive impairment that were higher than many unselected stroke populations, and our rates of impairment were also higher than those reported in other AF cohorts (226, 260-270). This might reflect the variability in methods used to diagnose pre-existing cognitive impairment, including different IQCODE thresholds.

Our finding that MTA is a common and prevalent finding in patients prior to stroke (31.6% of our cohort had grade 2 or higher) provides further evidence that this neuroimaging feature is important in the cognitive sequelae of AF-related ischaemic stroke and TIA. AF has been shown to be associated with lower hippocampal volumes and poorer memory and learning performance in stroke-free individuals, and patients with AF from the Alzheimer's Disease Neuroimaging Initiative (ADNI) had greater atrophy of their entorhinal cortex and medial temporal lobes, compared with those without AF (252, 277). The relationship between AF and global atrophy measures is less clear; whilst one study found that AF was associated lower brain volumes globally, others did not identify such an association, although this might reflect the younger age of these latter cohorts (252, 253, 278). This apparent association with MTA but not GCA is in keeping with our results,

and implicates AD pathology in the cognitive impairment associated with AF, a proposal for which there is supporting longitudinal and pathological (279-284) data. Proposed mechanisms by which AF might contribute to Alzheimer's disease pathology include β- and γ-secretase inhibition, perivascular amyloid clearance failures and tau phosphorylation, all of which might be induced by AF-related cerebral hypoperfusion (279). However, MTA can also be a feature of vascular pathology, and it might be that this is the dominant pathology in AF (285-288).

We also found an association with increasing WMH severity and pre-existing cognition. Although WMH in patients with AF might simply be due to age or a shared vascular risk factor profile (289), there is evidence to suggest that there is an independent association (290). Whilst WMH are associated with poorer cognitive performance (169), the data relating to cognitive impairment in AF and WMH is conflicting, with some studies showing no association (252, 253). We did not find an independent statistically significant association with imaging evidence of previous cortical infarcts, which might provide further evidence that embolism to the brain (either clinically overt or "silent") is not the only mechanism contributing to cognitive impairment in these patients. The lack of association between pre-existing cognitive impairment and other structural small vessel disease markers (MRI-visible perivascular spaces and cerebral microbleeds) is in keeping with data from other populations, which show inconsistent associations between cognitive impairment and these markers (3). The presence of both neurodegenerative and vascular pathologies support the argument that, in patients with ischaemic stroke, pre-existing dementia is a manifestation of "brain aging" (254) rather than due to one single pathological process; this is in contrast with the cognitive changes that occur prior to spontaneous intracerebral haemorrhage, where cerebral small vessel diseases (in particular, CAA) might be primarily responsible for the deficits observed (133, 191). The associations of IQCODE impairment with acute cognitive performance and later functional outcome might suggest that any future therapeutic strategies that address these measures will need to implemented early and prior to stroke in order to be effective.

Questions remain about how best to diagnose pre-existing cognitive impairment. The IQCODE has been used extensively and is sensitive to early cognitive changes (228). Our data provides more evidence that the IQCODE might be a useful and relevant tool in acute stroke, as it appears able to identify patients at risk of subsequent cognitive impairment and poorer functional outcomes 24 months after the index ischaemic event. Whilst it might seem counter-intuitive to use a questionnaire that does not require a direct assessment of the patient themselves, this is often useful in stroke where patients might be unable to respond to formal testing, for example due to aphasia or reduced consciousness. IQCODE-based estimates of cognition might prove more accurate than the potential overestimation of deficits resulting from acute patient testing (which can be influenced by intercurrent illness) and potential underestimation from formal dementia diagnoses. The association of IQCODE-defined cognitive impairment with recognised neurodegenerative and vascular neuroimaging markers suggests that this is indeed reflective of significant underlying pathology, as do its associations with later cognitive and functional outcome measures.

The strengths of this study include its large size and its prospective multicentre design. We also consider a wide range of structural markers associated with cerebral small vessel disease and neurodegeneration. However, there are also some limitations. Whilst the study imaging protocol required standardised sequences, there was still variability in how these sequences were obtained, as well as the MRI machines used by each centre, and this could influence our imaging rating. We also acknowledge that the IQCODE threshold used might not be equivalent to dementia and that a range of thresholds have been used in the past; the IQCODE has not been validated for pre-stroke impairment against a formal diagnosis of dementia, and we too were unable to comment on this in our study. Despite this, we would argue that cognitive impairment at the level identified by the IQCODE is still of relevance given that it is able to predict future outcomes, and

the lack of formal diagnoses of dementia prior to the index ischaemic event is in keeping with what is observed in this clinical setting.

In this comprehensive imaging description of the factors associated with pre-existing cognitive impairment in cardioembolic stroke and TIA, we report that pre-existing cognitive impairment is common, and associated with imaging markers of cerebral small vessel disease and neurodegeneration, as well as immediate cognitive performance and poorer functional outcomes at 24 months. We also provide evidence that the IQCODE might be useful as an acute tool in ischaemic stroke and TIA, by identifying those likely to have worse clinical outcomes. Future work validating the IQCODE in this context, together with further investigation of the factors that contribute to brain resilience and whether this can be influenced after ischaemic injury, is needed.

2.7 Small vessel predictors of cognitive performance after cardioembolic ischaemic stroke or TIA

This section was submitted for publication in October 2018 (Appendix 2, submitted manuscript III), and completed in collaboration with the authors listed.

2.7.1 Introduction

Post-stroke dementia is common but has heterogenous mechanisms that are not fully understood. Early post-stroke dementia (within 6 months) is associated with factors relating to brain resilience and the index stroke lesion, whereas delayed-onset post-stroke dementia is more associated with cerebral small vessel diseases (291). Whilst dementia after ischaemic intracerebral events (stroke or TIA) is associated with white matter hyperintensities, lacunes and cortical atrophy (292, 293), markers of CAA are associated with dementia after intracerebral haemorrhage (123).

The natural history of post-stroke dementia is further complicated by the fact that cognitive performance immediately after a stroke might not be representative of later cognition, as performance might improve; this occurs both acutely, where the initial assessment might be influenced by delirium, but also over longer time periods (294-298). The Montreal Cognitive Assessment (MoCA) appears to be a sensitive cognitive screen for identifying these changes (299), and there has been recent interest in identifying the characteristics of so-called “reverters”, who demonstrate improvements in their cognitive performance over time (299-302).

We investigated cognitive trajectory in patients with AF-related ischaemic stroke or TIA. Our objectives were: (1) to describe the changes in MoCA that occur between acute (immediately after the ischaemic event) and 12 month assessment, (2) to investigate the clinical and radiological associations of MoCA-defined cognitive impairment at 12

months, and (3) to describe the clinical and radiological features associated with a failure to improve cognitively 12 months after stroke.

2.7.2 Methods

2.7.2.1 Participants

This is another subgroup analysis of the CROMIS-2 AF study; the details relating to this study are provided in Section 2.6.2.1. We excluded patients with a formal diagnosis of dementia or cognitive impairment at study entry. We also excluded patients with IQCODE-defined cognitive impairment (IQCODE score > 3.3) prior to study entry (see Section 2.5.2.1 for further details) (229). Additionally, all patients were required to have 12 month MoCA data in order to be included (Figure 2.7.1). We compared the characteristics of eligible patients with and without 12 month MoCA data in order to review for selection bias.

2.7.2.2 Cognitive measures

The “acute” MoCA was collected immediately after the index ischaemic event. All participating centres were invited to collect additional MoCA data at 12 months (“12 month MoCA”) following study entry; twenty centres agreed to contribute to this substudy. A MoCA score <26 was used to define cognitive impairment (274). “Reverters” were defined as patients with an acute MoCA score <26, who demonstrated an improvement of ≥2 points at 12 months, in accordance with previously published work (299, 300); patients with an acute MoCA score <26 who did not show this improvement were defined as “non-reverters”.

2.7.2.3 Imaging Acquisition and Analysis

The methods for imaging acquisition and the rating of structural markers of SVD are described in Section 2.6.2.2. The composite CAA and SVD scores were calculated as described in Section 2.3.8 and 2.3.9 respectively.

The presence of an acute DWI lesion was confirmed if a lesion was bright on the B1000 and dark on the corresponding ADC map; the side of the lesion, presence of single or multiple acute lesions, and evidence of cortical involvement were recorded (Duncan Wilson). Evidence of haemorrhagic transformation was rated using the ECASS classification (303) using T2*-GRE sequences (Duncan Wilson). The occurrence of further intracerebral events was independently adjudicated by Duncan Wilson and a Professor of Vascular Neurology (David Werring).

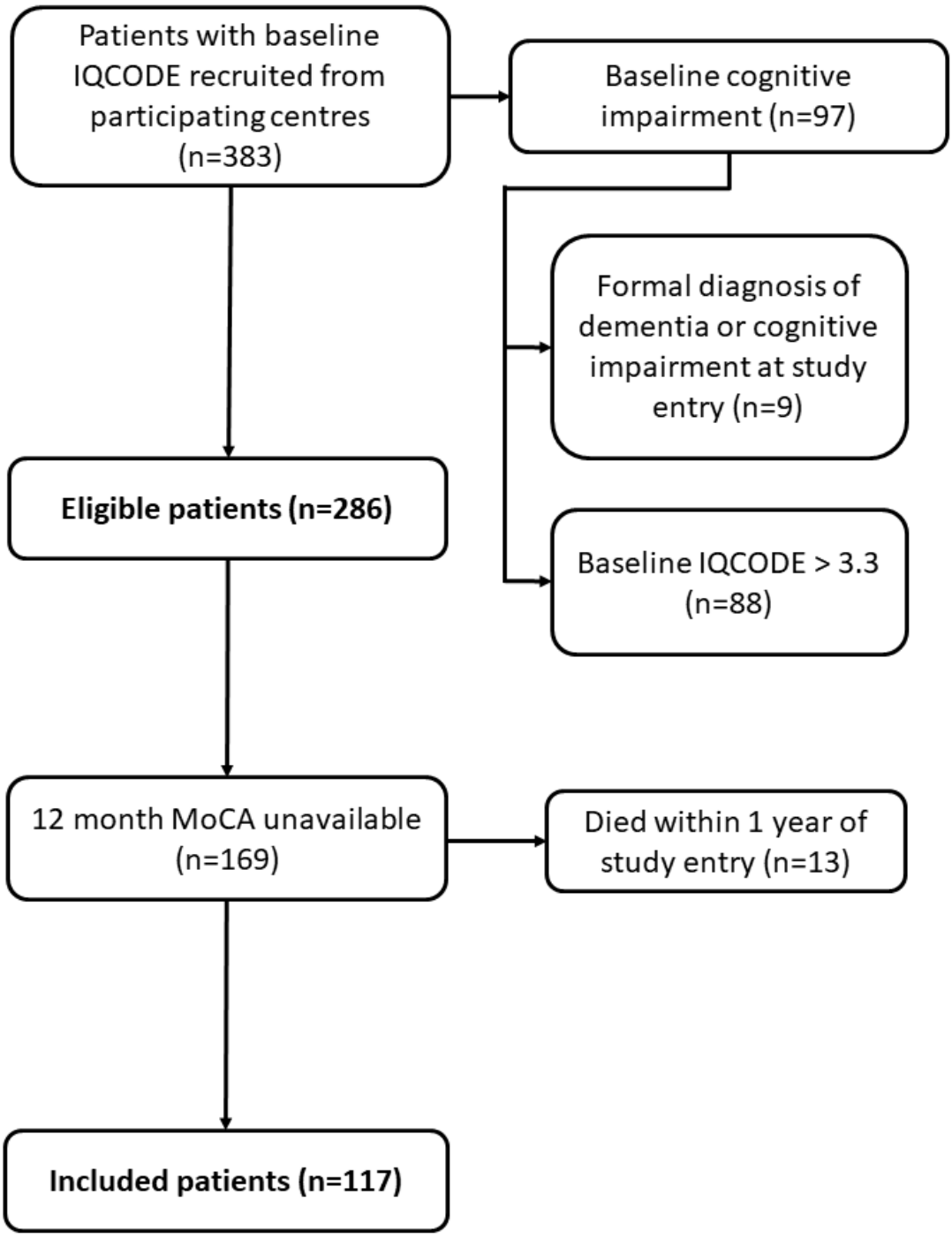
2.7.2.4 Statistics

Statistical analysis was performed by the candidate using Stata (Version 11.2). We compared baseline clinical, demographic and imaging findings in patients with and without MoCA-defined cognitive impairment at 12 months, and for reverters compared with non-reverters. For all continuous variables, data were reviewed for normality, and if normally distributed the independent t-test was used. If variables were ordinal or not normally distributed, the non-parametric Mann Whitney U test was used. Chi-squared or Fisher's exact tests were used for categorical variables. Performances of acute and 12 month MoCA were compared using paired t-tests (mean scores) or McNemar's test (proportion impaired).

The results of univariate comparisons were used to identify variables for inclusion in multivariable logistic regression models; all variables with $p<0.20$ were included in the

adjusted analyses except for situations where variables both described the same phenomenon (for example, clinical history of previous ischaemic events and imaging evidence of a previous cortical infarct). Adjusted models considered only a single neuroimaging marker at a time. Given that these analyses were exploratory, we did not make an adjustment for multiple testing.

Figure 2.7.1: Description of the study population



2.7.3 Results

2.7.3.1 Participants

383 patients were recruited from the twenty sites participating in the MoCA substudy; 286 patients were eligible for this substudy, of whom 117 had both baseline and 12 month cognitive data available (Figure 2.7.1). We compared the characteristics of eligible patients without 12 month MoCA data and the included patients in order to review for selection bias (Table 2.7.1). The included patients were less likely to have a diagnosis of diabetes mellitus (9.5% vs 19.5%, p=0.02), less likely to have known AF prior to study entry (23.9% vs 36.3%, p=0.031), and had a higher educational age (mean 16.8 vs 16.0 years, p=0.03). Included patients also had lower admission NIHSS at study entry (median 3.5 vs 5.5, p=0.02), higher acute MoCA scores (median 25 vs 23, p=0.00091) and lower mRS scores at discharge (IQR 0 to 2 vs 1 to 3, p=0.0321); there were no other differences in baseline demographic or clinical variables.

2.7.3.2 Comparison of acute and 12 month MoCA performance

Acute MoCA data was available for 114 patients with 12 month MoCA data (Figure 2.7.2). The median time to acute MoCA assessment was 4 days (IQR 2 to 8 days; range 0 to 31 days). Overall, there was an improvement at 12 months compared with acute performance (mean difference 1.69 points where maximum score is 30, p<0.00001; Table 2.7.2). Scores improved across all subdomains except for attention (which showed a deterioration) and were statistically significant for visuo-executive function (mean difference 0.23 points, p=0.0470), abstraction (mean difference 0.14 points, p=0.0176) and delayed recall (mean difference 0.62 points, p=0.0002).

We also considered whether the proportion of participants impaired across domains (defined as scoring less than full marks) changed with time (Table 2.7.3). Fewer patients demonstrated MoCA impairments at 12 months (51.3% vs 57.9%, p=0.0719), and there

were lower proportions of impaired participants across all domains; this was statistically significant for language (48.7% vs 59.7%, p=0.0269), and abstraction (22.2% vs 31.6%, p=0.0233).

2.7.3.3 Clinical and imaging associations of cognitive impairment 12 months following index ischaemic event

Amongst those with available 12 month MoCA data (n=117), 51.3% (n=60) had an abnormal MoCA score (<26) at 12 months; of these, 81.7% (n=49) had an abnormal MoCA acutely. When comparing those with and without MoCA-defined cognitive impairment at 12 months, those with impairment were older (mean age 75.6 years vs 70.5 years, p=0.0022), had fewer years of education (mean 15.9 years vs 17.7 years, p=0.0077), had a higher admission NIHSS (median score 5.5 vs 2, p=0.0060), lower acute MoCA score (median 22 vs 27, p<0.00001) and higher discharge mRS (median score 2 vs 1, p=0.0004). In a multivariable logistic regression analysis including these variables, only acute MoCA score remained associated with MoCA impairment at 12 months (per point increase, OR 0.73, 95% CI 0.59 to 0.91, p=0.005); in a multivariable model excluding acute MoCA score, discharge mRS (per point increase, OR 1.91, 95% CI 1.07 and 3.44, p=0.029) was the only variable that remained associated with 12 month MoCA score.

Patients with cognitive impairment at 12 months had higher grades of pvWMH (IQR 0 to 1 vs 0 to 0, p=0.0545) and had higher CAA scores (median score 0.5 vs 0, p=0.0005). There were no differences between the two groups in the imaging features of the index ischaemic lesion (presence of acute DWI lesion at study entry, side of index lesion, presence of multiple index lesions on DWI, presence of a cortical lesion, evidence of haemorrhagic transformation). In adjusted analyses (adjusted for age, educational age, discharge mRS and acute MoCA score), cognitive impairment at 12 months remained

associated with CAA score (per point increase, OR 4.09, 95% CI 1.36 to 12.33), p=0.012) but not pvWMH grade (OR 1.15, 95% CI 0.54 to 2.44, p=0.725).

2.7.3.4 MoCA trajectory

In this cohort, 66 patients had an acute MoCA score below 26; of these, 59.1% (n=39) were “reverters” (Figure 2.7.3). Non-reverters had higher acute MoCA scores (median 24 vs 21, p=0.0002) and lower 12 month MoCA scores (median 23 vs 25, p=0.0008); there were no other clinical or demographic differences between the two groups.

The imaging characteristics of reverters and non-reverters are shown in Table 2.7.4. Non-reverters had lower baseline pvWMH grade (IQR 0 to 0 vs 0 to 1, p=0.0752), but higher CSO-PVS grade (median grade 2 vs 1, p=0.0306), and were more likely to have CMBs (22.2% vs 2.6%, p=0.016) and in particular, strictly lobar CMBs (14.8% vs 0.0%, p=0.024). Non-reverters also had a higher composite SVD (mean 0.88 vs 0.27, p=0.0046) and CAA (mean 0.80 vs 0.25, p=0.0007) scores. In unadjusted logistic regression analyses (Table 2.7.5), non-reversion remained positively associated with CSO-PVS grade (per grade increase, OR 1.83, p=0.029), cerebral microbleed presence (OR 10.86, p=0.032), SVD score (per point increase, OR 2.91, p=0.015) and CAA score (per point increase, OR 6.71, p=0.001), and negatively associated with the presence of multiple lesions at study entry (OR 0.11, p=0.040). Similar associations were observed in analyses adjusted for MoCA score (Table 2.7.5).

Table 2.7.1: Baseline characteristics of included and excluded patients

Percentage values were calculated using the total number of patients for whom data was available as the denominator. p values are from independent t-tests (age, educational age, discharge mRS), Mann Whitney U test (NIHSS, acute MoCA score), Fisher's exact test (heart failure) or chi-squared tests (remainder).

	Included (n=117)	**Excluded (n=168)**	**p value**
Age, years , mean (SD)	73.1 (9.1)	74.2 (10.7)	0.3757
Sex, female, n (%)	45 (38.5)	69 (40.8)	0.688
Hypertension, n (%)	60 (52.2)	100 (59.2)	0.243
Hypercholesterolaemia, n (%)	51 (44.4)	83 (49.7)	0.376
Diabetes mellitus, n (%)	11 (9.5)	33 (19.5)	0.021
Smoking			
Never	51 (44.0)	77 (45.6)	0.960
Ex-smoker	54 (46.6)	77 (45.6)	
Current smoker	11 (9.5)	15 (8.9)	
Heart failure, n (%)	4 (3.5)	9 (5.4)	0.569
AF prior to study entry, n (%)	28 (23.9)	61 (36.3)	0.027
Educational age, years, mean (SD)	16.8 (3.5)	16.0 (2.5)	0.0288
NIHSS, median (IQR)	3.5 (2 to 9)	5.5 (2 to 11)	0.0210
Acute MoCA score, median (IQR)	25 (21 to 27)	23 (18 to 26)	0.0091
Discharge mRS, median (IQR)	1 (0 to 2)	1 (1 to 3)	0.0321
Further intracerebral event within 12 months of study entry, n (%)	6 (5.1)	9 (5.3)	0.941

Figure 2.7.2: Distribution of acute and 12 month MoCA scores

Each patient is shown by a single diamond; the data has been jittered to show individual points. The line of equality is shown in red.

Table 2.7.2: Comparison of MoCA performance (scores) acutely and at 12 months

Acute MoCA assessed median 4 days following ischaemic event. p values are from paired t-tests.

	Maximum achievable score	Acute MoCA, mean score (SD)	12 month MoCA, mean score (SD)	Mean difference (95% CI)	p value
Overall	30	23.55 (4.95)	25.25 (3.88)	1.69 (1.03 to 2.36)	p<0.00001
Visuo-executive	5	3.77 (1.38)	4.00 (1.13)	0.23 (0.00 to 0.45)	0.0470
Naming	3	2.75 (0.61)	2.82 (0.49)	0.07 (-0.05 to 0.19)	0.2399
Attention	6	4.98 (1.49)	4.70 (1.60)	-0.28 (-0.05 to 0.61)	0.0964
Orientation	6	5.58 (1.06)	5.71 (0.73)	0.13 (-0.05 to 0.32)	0.1628
Language	3	2.11 (0.91)	2.25 (0.96)	0.13 (-0.05 to 0.32)	0.1591
Abstraction	2	1.58 (0.68)	1.72 (0.59)	0.14 (0.02 to 0.26)	0.0176
Delayed recall	5	2.34 (1.62)	2.96 (1.53)	0.62 (0.31 to 0.94)	0.0002

Table 2.7.3: Comparison of MoCA performance (impairment) acutely and at 12 months

Acute MoCA assessed median 4 days following ischaemic event.

Impairment was defined as scoring less than full marks in a given domain; MoCA impairment was defined as previously (score <26). Percentage values were calculated using the total number of patients for whom data was available as the denominator. p values are from McNemar's tests.

	Maximum achievable score	Acute MoCA, impaired n (%)	12 month MoCA, impaired n (%)	p value
Overall	30	66 (57.9)	60 (51.3)	0.0719
Visuo-executive	5	72 (63.2)	69 (59.0)	0.2888
Naming	3	22 (19.3)	17 (14.5)	0.3173
Attention	6	61 (53.5)	54 (46.2)	0.1228
Orientation	6	25 (21.9)	24 (20.5)	0.5050
Language	3	68 (59.7)	57 (48.7)	0.0269
Abstraction	2	36 (31.6)	26 (22.2)	0.0233
Delayed recall	5	102 (89.5)	98 (83.8)	0.1444

Figure 2.7.3: Distribution of acute and 12 month MoCA scores for reverters and non-reverters

Each patient is shown by a single symbol, as indicated by the key; the data has been jittered to show individual points. The line of equality is shown in red.

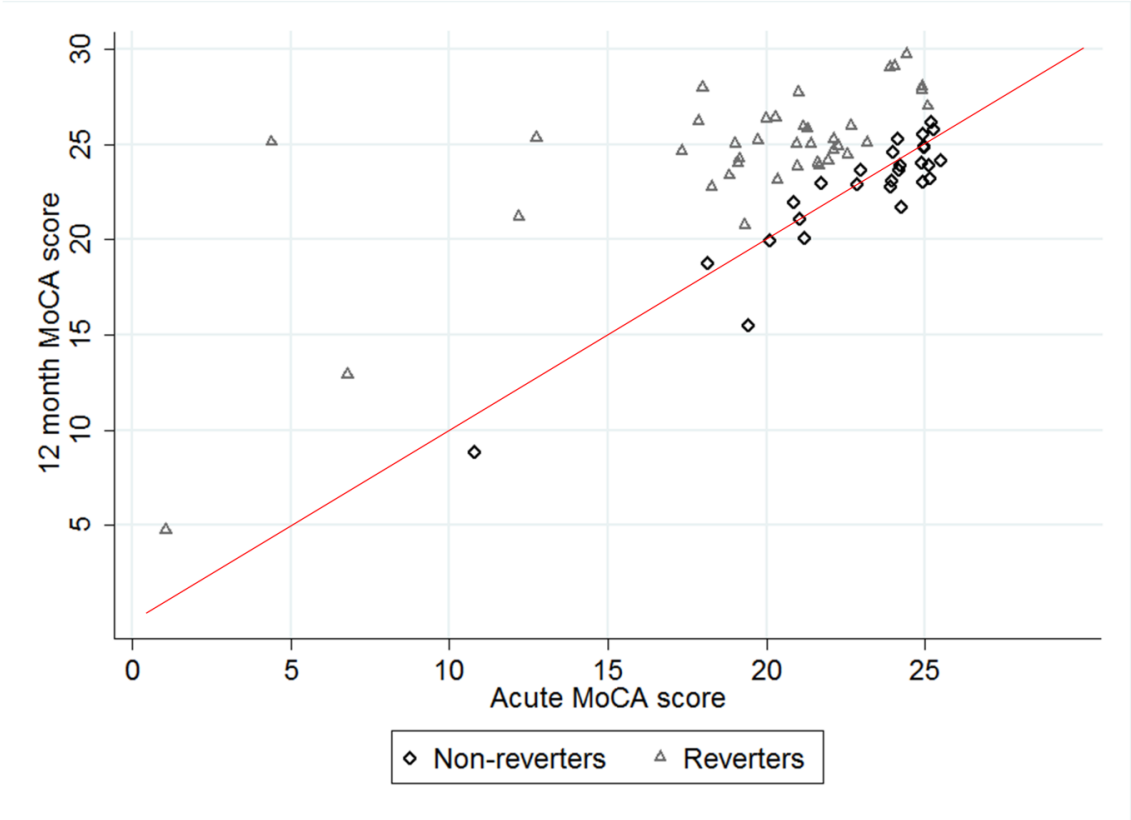


Table 2.7.4: Comparison of imaging characteristics of reverters vs non-reverters

Percentage values were calculated using the total number of patients for whom data was available as the denominator. p values are from Mann Whitney U tests (where median and IQR are given), chi-squared tests (acute DWI lesion at study entry) or Fisher's exact test (remainder).

		Reverters	Non-Reverters	p value
n (%)		39 (59.1)	27 (40.9)	-
Structural imaging markers at study entry				
Imaging evidence of previous cortical infarct, n (%)		3 (7.7)	4 (14.8)	0.432
Lacunes, presence, n (%)		5 (12.8)	5 (20.8)	0.485
pvWMH grade, median (IQR)		0 (0 to 1)	0 (0 to 0)	0.0752
dWMH grade, median (IQR)		1 (0 to 1)	0 (0 to 1)	0.3217
CSO-PVS grade, median (IQR)		1 (1 to 2)	2 (1 to 3)	0.0306
BG-PVS grade, median (IQR)		1 (1 to 1)	1 (1 to 1)	0.1221
MTA grade, median (IQR)		1 (0 to 1)	1 (0 to 1)	0.7561
GCA grade, median (IQR)		1 (0 to 1)	1 (0 to 1)	0.9943
CMB, presence, n (%)		1 (2.6)	6 (22.2)	0.016
Strictly lobar CMB, n (%)		0 (0.0)	4 (14.8)	0.024
Composite SVD score, median (IQR)	Median (IQR)	0 (0 to 0)	1 (0 to 1)	0.0046
	Mean (SD)	0.27 (0.61)	0.88 (0.93)	
Composite CAA score, median (IQR)	Median (IQR)	0 (0 to 0.5)	1 (0 to 1)	0.0007
	Mean (SD)	0.25 (0.44)	0.80 (0.62)	
Imaging features of index ischaemic event				
Acute DWI lesion at study entry, n (%)		32 (82.1)	19 (76.0)	0.557
Side of index lesion, n (%)				
Left		11 (35.5))	6 (31.6)	0.249
Right		16 (51.6)	13 (68.4)	
Bilateral		4 (12.9)	0 (0.0)	
Presence of multiple lesions, n (%)		11 (34.4)	1 (5.3)	0.020
Cortical lesion, n (%)		21 (65.6)	14 (73.7)	0.756
Evidence of haemorrhagic transformation, n (%)		8 (20.5)	2 (8.7)	0.298

Table 2.7.5: Unadjusted and adjusted logistic regression analyses for predictors of non-reversion

Each model considered a single imaging marker at a time.

	Unadjusted		Adjusted for acute MoCA score	
	OR (95% CI)	**p value**	**OR (95% CI)**	**p value**
pvWMH (per grade increase)	0.41 (0.12 to 1.35)	0.143	-	-
CSO-PVS (per grade increase)	1.83 (1.06 to 3.15)	0.029	1.96 (1.05 to 3.66)	0.035
CMB (presence)	10.86 (1.22 to 96.34)	0.032	9.36 (0.92 to 95.34)	0.059
SVD score (per point increase)	2.91 (1.23 to 6.88)	0.015	2.47 (1.02 to 6.00)	0.046
CAA score (per point increase)	6.71 (2.10 to 21.50)	0.001	6.70 (1.88 to 23.98)	0.003
Presence of multiple lesions at study entry	0.11 (0.02 to 0.90)	0.040	0.11 (0.01 to 1.01)	0.051

2.7.4 Discussion

MoCA-defined cognitive impairment at 12 months is common, observed in approximately half of our cohort, and associated with factors to relating to brain resilience (age, educational age) and stroke severity (acute MoCA score, NIHSS, discharge mRS), as well as increases in a composite CAA score. Overall, we found that MoCA performance at 12 months improves compared with acute performance, and we show that the presence of structural imaging markers of small vessel disease (CSO-PVS, cerebral microbleeds, composite SVD and CAA scores) is associated with non-reversion.

Our use of composite scores for the two most common small vessel diseases provides new perspectives on the small vessel mechanisms which might underlie post-stroke dementia. Composite scores are hypothesised to better reflect overall pathological burden and have shown associations with a number of clinical measures including cognitive performance (180-183, 187). In our study, we observed an independent association of 12 month cognitive performance with the composite CAA score. This score includes non-haemorrhagic markers of CAA such as CSO-PVS and WMH which do not feature in the current diagnostic criteria for CAA (57), and which might have more relevance in non-haemorrhagic patient populations. The association of CAA with dementia following intracerebral haemorrhage has been described (123), as have associations between strictly lobar microbleeds and executive function in patients with ischaemic stroke or TIA (304). Whilst our finding that CAA score is associated with 12 month MoCA impairment should be interpreted with caution, given the low prevalence of haemorrhagic markers and the relatively small size of the cohort, it might provide further evidence that imaging markers of CAA are associated with cognitive performance beyond intracerebral haemorrhage, a finding already observed in non-haemorrhagic memory clinic populations (135, 159).

Our observation that overall cognitive performance can improve with time following an ischaemic event is in keeping with data from previous studies (294-298), as are the significant domain specific improvements in visuo-executive function, abstraction and delayed recall (299, 300). As well as improvements in raw scores, we also found that levels of impairment were lower at 12 months for most domains. However, we did note that for a number of domains, whilst raw scores improved significantly, patients remained in the impaired range (as we defined it). This highlights the difficulties in quantifying deficits when considering individual domains.

We also describe the characteristics of patients with impaired acute performance who demonstrate an improvement of two or more points - so-called “reverters” (299, 300) – and those who do not. Whilst use of the term “reverter” has been criticised for suggesting that cognitive performance returns to normal (300), it is useful as a standardised method for defining improvement. Whilst we did not find any clinical or demographic differences between reverters and non-reverters (except for those relating to MoCA scores), there were imaging differences. Non-reverters appeared to have more evidence of small vessel disease (CSO-PVS, cerebral microbleeds, and higher composite SVD and CAA scores), and were less likely to have had multiple acute DWI lesions at study entry. As discussed above, the association between both multiple lesions and lower acute MoCA scores amongst the reverters might suggest that multiple lesions are more likely to result in an acute reversible cognitive impairment – although the acute disturbance in this cohort does not seem to be typical for delirium, given the lack of attentional improvement with time. Replication of this work in larger cohorts will be important for confirming and better quantifying these observations.

The strengths of this study are its multicentre prospective design, and the detailed clinical and radiological descriptions available for the study participants. However, there are also some limitations. Firstly, only a subset of centres collected 12 month MoCA data, and

even within these centres MoCA data was not collected for all potentially eligible participants. The excluded patients had more comorbidities, lower educational age, more severe ischaemic events (as defined by NIHSS and discharge mRS) and had lower acute MoCA scores, all of which are associated with poorer cognitive outcome at 12 months. Given this, the cognitive performance of our cohort might be better than expected and not representative of all cardioembolic ischaemic stroke or TIA cohorts. We note that group sizes for some analyses are small and the prevalence of haemorrhagic markers in our cohort was low; in view of this, these results should be interpreted cautiously. Finally, the MoCA has some intrinsic limitations, including the fact that it is primarily a screening tool and thus likely underestimates the severity and breadth of cognitive impairment that result from stroke (305, 306). Nevertheless, the positive findings and associations we describe suggest that the MoCA might be a useful tool for monitoring for change over time.

We conclude that cognitive impairment 12 months following an ischaemic event is common, and that structural imaging features of small vessel disease appear associated both with deficits and a lack of improvement at 12 months. Further work that clarifies the role of small vessel diseases in this context will be essential for refining future cognitive rehabilitation strategies.

3 Small vessel diseases and clinical outcomes following spontaneous intracerebral haemorrhage

Spontaneous ("primary") ICH is associated with significant morbidity and mortality, and its incidence continues to increase worldwide (5, 20, 49, 307-309). Most research on outcomes following ICH has focussed on short term prognosis, reflecting the high rates of death associated with this stroke subtype (20, 309). However, perceptions of ICH are changing. Acute interventions including active blood pressure management, the reversal of anticoagulation, and prompt neurosurgical referral might improve immediate prognosis in patients with ICH (310). There are also new pharmacological strategies being tested in ICH, such as tranexamic acid, which might improve prognosis in the short term (311). This "active" immediate management of ICH is focussed on treating factors associated with early mortality; if these interventions are successful and there are consequently more ICH survivors, a better understanding of the characteristics that influence subsequent outcomes will be essential for guiding management beyond the acute stage.

This section describes two projects which consider long-term (3 year) outcomes in patients with ICH. The first project investigates the risk of recurrent ICH and later cerebral ischaemic events in patients initially presenting with ICH, and the role of ICH location (as a surrogate for the underlying SVD) in determining this risk. The second project considers the factors (including CT markers of SVD) that influence mortality over this time period, and in particular whether the effect of these baseline measures on mortality changes with time.

3.1 Long-term stroke risk following spontaneous intracerebral haemorrhage

3.1.1 Introduction

As described, the incidence and prevalence of spontaneous ICH is continuing to increase worldwide (5). ICH is associated with high rates of mortality (with 1 year and 5 year survival estimated at 46% and 29% respectively (20)), and consequently data on ICH survivors are limited, in particular, data on subsequent stroke events (20, 24-26). This leads to management dilemmas in patients who are at risk of ischaemic vascular events (involving the brain, heart or peripheral vasculature) or thromboembolic disease, and who may benefit from treatment with antiplatelet or anticoagulant medications.

One baseline feature which may help identify patients at higher risk of subsequent stroke events is whether they have imaging evidence of small vessel disease. Lobar ICH has a higher recurrence rate (20), which is thought to reflect its association with the bleeding-prone CAA (2, 22). DPA is thought to be responsible for non-lobar or “deep” ICH and is associated with cardiovascular risk factors and lacunar infarction (2), and thus may present a greater ischaemic risk together with lower rates of ICH recurrence. It has been suggested that, given their increased ICH risk, patients with lobar ICH should not be offered anticoagulation, even those with AF (which confers a particular high ischaemic stroke risk) (21, 312). However, recent data challenges this view; an individual patient data meta-analysis (23), which included 1012 patients who resumed treatment with oral anticoagulant therapy following spontaneous ICH, found that resumption was associated with reduced mortality and all-cause stroke incidence, as well as more favourable outcomes, at 1 year. This suggests that the “early” (i.e. up to 1 year) risk of ischaemic events may be underestimated in these patients and that they may benefit from anticoagulation at this stage; what is not clear is whether this remains the case in the longer term, beyond 1 year.

The study aims to provide new data on stroke risk following spontaneous ICH in a large cohort of ICH survivors. The specific objectives are: (1) to describe the incidence of recurrent ICH and cerebral ischaemic events in the longer term (up to 3 years) following ICH, and (2) to evaluate the influence of ICH location, as a surrogate marker for the underlying small vessel disease, on stroke outcomes.

3.1.2 Methods

3.1.2.1 Participants

We included patients recruited to a prospective multicentre observational cohort study of symptomatic adults (aged over 18 years) with imaging confirmed ICH (CROMIS-2 ICH; https://clinicaltrials.gov; NCT02513316); full details of the study protocol have been published previously (227) and described earlier in this thesis (Section 2.5.2.1). The study was approved by the National Research Ethics Service (IRAS reference 10/H0716/61), and written informed consent was obtained for each patient.

Patients were considered to have pre-existing cognitive impairment if they had a formal diagnosis of dementia or cognitive impairment at study entry, or if they scored more than 3.3 on the 16-item IQCODE, in accordance with previous data (229) and as described earlier in this thesis (Section 2.5.2.1). *APOE* genotype was established from peripheral blood samples (by Isabel Hostettler, Clinical Research Associate); the method for this has been previously described (313).

3.1.2.2 Outcomes

For the first 6 months after the index event, outcomes were collected using multiple ascertainment methods, as detailed in the previously published study protocol (227). Briefly, these methods included postal questionnaires sent to patients and their general practitioners, and notifications from NHS Digital (previously the Health and Social Care

Information Centre) (227). Outcome data from 6 months to 3 years were compiled from notifications from NHS Digital. Hospital episode statistics for all admitted patient care (APC) events were reviewed using the NHS Digital HES Data Dictionary for APC episodes (314). An “admission” was defined as one or more individual episodes, which ended with the patient being discharged to a “home destination” (DISDEST codes 19, 29, 30, 49, 50, 54, 65, 85) or hospice (DISDEST code 88), or with the death of the patient (DISDEST code 79). The primary diagnosis (DIAG_01 code) was determined using the online version of the World Health Organisation International Statistical Classification of Diseases and Related Health Problems (315). A cerebrovascular event was defined as an admission due to a cerebral ischaemic event (G459, I632, I633, I634, I635, I638, I639, I663), ICH (I610, I611, I612, I614, I615, I616, I618, I619), other non-traumatic intracranial bleeding events (I609, I620, I629), or unspecified stroke event (I64X, I678). Outcome events were diagnosed locally and not adjudicated centrally.

The outcomes of interest were occurrence of a cerebral ischaemic event (either stroke or TIA) or a further ICH following study entry. Patients were censored at the time of their first cerebrovascular event or date of death. If this data was unavailable, they were censored either 3 years following their index event, or at the time of the study’s last notification from NHS Digital (31/03/2017); the earlier date of these two was used in these cases.

3.1.2.3 Imaging

Brain CT imaging was acquired acutely at the time of the index event as part of the patient’s routine clinical care. All CT imaging was rated by Duncan Wilson (Clinical Research Associate). Haematoma location was classified using the CHARTS scale (13) as lobar (including convexity subarachnoid haemorrhage), deep (involving the basal ganglia or thalamus), cerebellar or brainstem. Non-lobar was defined as the presence of either deep or brainstem haemorrhage; cerebellar haemorrhage was excluded from this

definition as this does not have a clear small vessel disease association. CT images were also rated for the presence of lacunes, which were defined in accordance with STRIVE criteria (7). WMH were rated on CT images using the Van Swieten score; the highest scores for anterior and posterior regions were combined in order to generate a "total" score (range 0 to 4) (316).

3.1.2.4 Statistics

Statistical analysis was performed by the candidate using Stata (Version 11.2). Univariable Cox regression was used to compare clinical and imaging variables associated with the occurrence of an outcome of interest; this was used due to the variation in length of follow up for each patient. Multivariable Cox regression analysis was then performed; adjustments were made for all variables with $p<0.10$ in univariable analyses, in addition to the primary variable of interest (ICH location). The proportional-hazards assumption test based on Schoenfeld residuals was applied to all Cox models (univariable and multivariable). Univariable and multivariable competing risk analyses (using the Fine-Gray subdistribution hazard model) were also performed; subdistribution hazard ratios (SHR) are provided.

3.1.3 Results

All 1094 patients recruited to CROMIS-2 ICH were included (baseline characteristics are shown in Table 3.1.1); 447 (40.9%) were lobar ICH, 546 (50.0%) were deep, 65 (6.0%) were cerebellar, and 34 (3.1%) occurred in the brainstem. Follow up was for a total of 2390.72 patient-years (median 3.00 years, IQR 1.48 to 3.00 years).

3.1.3.1 Recurrent ICH events

There were 45 recurrent ICH events (absolute event rate 1.88 per 100 patient-years, 95% CI 1.41 to 2.52 per 100 patient-years); 35 were in patients whose index event was

lobar (n=447, 929.11 patient-years follow up; absolute event rate 3.77 per 100 patient-years, 95% CI 2.70 to 5.24 per 100 patient-years), and 9 in patients presenting with non-lobar ICH (n=580, 1311.19 patient-years follow up; absolute event rate 0.69 per 100 patient-years, 95% CI 0.36 to 1.32 per 100 patient-years). The absolute event rate for patients presenting with deep ICH was 0.73 per 100 patient-years (95% CI 0.38 to 1.41 per 1000 patient-years; 9 events in 1227.96 patient-years) and 0.69 per 100 patient-years for those presenting with cerebellar ICH (95% CI 0.01 to 4.92 per 1000 patient-years; 1 event in 144.42 patient-years); there were no recurrent ICH events in patients presenting with brainstem ICH (n=34, 83.23 patient years).

In univariable Cox regression analyses (Table 3.1.2), recurrent ICH events were associated with increasing age, and with a history of previous cerebral ischaemic events, ICH prior to study entry, and antiplatelet use prior to study entry. There were also associations with the severity of white matter disease (as measured by increasing Van Swieten score) and lobar ICH location on baseline imaging (Figure 3.1.1). Similar results were observed in competing risk regression for recurrent ICH events (Table 3.1.2) in analyses where occurrence of an ischaemic event or death was the competing risk, including a similar association with lobar ICH location as baseline (Figures 3.1.2 and 3.1.3).

Multivariable Cox regression (including all six variables listed above, in addition to *APOE* ε2 genotype; Table 3.1.3) found that lobar ICH location at presentation remained associated with subsequent ICH occurrence (HR 8.70, 95% CI 3.29 to 23.03, p<0.0001). A history of cerebral ischaemic events (HR 2.32, 95% CI 1.12 to 4.79, p=0.023), ICH (HR 3.87, 95% CI 1.16 to 12.93, p=0.028), and antiplatelet use (HR 2.62, 95% CI 11.31 to 5.24, p=0.006) prior to the index event were also associated with subsequent ICH occurrence, as was increasing Van Swieten score (HR 1.27, 95% CI 1.01 to 1.59, p=0.041). Multivariable completing risk analyses with cerebral ischaemic events as the competing risk and including the same variables as the adjusted Cox regression, showed

similar results (Table 3.1.3): lobar ICH location remained associated with recurrent ICH events (SHR 8.55, 95% CI 3.28 to 22.28, p<0.0001), as did a history of previous ICH (SHR 3.70, 95% CI 1.05 to 12.99, p=0.042), prior antiplatelet use (SHR 2.55, 95% CI 1.25 to 5.19, p=0.010) and increasing Van Swieten score (per point increase, SHR 1.26, 95% CI 1.00 to 1.59, p=0.051). Similar results were also obtained using death as a competing event (Table 3.1.3).

3.1.3.2 Cerebral ischaemic events

There were 70 cerebral ischaemic events (absolute event rate 2.93 per 100 patient-years, 95% CI 2.32 to 3.70 per 100 patient-years), of which 29 occurred in patients presenting with lobar ICH (absolute event rate 3.12 per 100 patient-years, 95% CI 2.17 to 4.49 per 100 patient-years) and 39 in patients with non-lobar ICH (absolute event rate 2.97 per 100 patient-years, 95% CI 2.17 to 4.07 per 100 patient-years). The absolute event rate for patients presenting with deep ICH was 3.01 per 100 patient-years (95% CI 2.18 to 4.16 per 100 patient-years; 37 events), 1.38 per 100 patient-years for those presenting with cerebellar ICH (95% CI 0.35 to 5.54 per 100 patient-years; 2 events) and 2.40 per 100 patient-years for those presenting with brainstem ICH (95% CI 0.60 to 9.61 per 100 patient-years; 2 events).

In univariable analyses (Table 3.1.4), subsequent cerebral ischaemic events were associated with increasing age, hypercholesterolaemia, AF, history of previous cerebral ischaemic events, anticoagulant use prior to ICH, and increasing Van Swieten score; there was no association with ICH location (Figure 3.1.4). Univariable competing risk regression for subsequent ischaemic events with occurrence of recurrent ICH or death as the competing risk found similar results (Table 3.1.4, Figures 3.1.5 and Figure 3.1.6).

Multivariable Cox regression (Table 3.1.5) found significant associations with a history of previous ischaemic events (HR 2.14, 95% CI 1.21 to 3.77, p=0.009) only. There was

no association with ICH location (HR 1.00, 95% CI 0.58 to 1.71, p=0.994). Similar results were seen in multivariable completing risk analyses with both recurrent ICH and death as the competing event (Table 3.1.5).

Table 3.1.1: Baseline characteristics

Percentage values were calculated using the total number of patients for whom data was available as the denominator.

	All
n	1094
Age, years, mean (SD)	73.3 (12.5)
Sex, male, n (%)	628 (57.4)
Hypertension, n (%)	718 (66.7)
Hypercholesterolaemia, n (%)	467 (44.0)
Diabetes mellitus, n (%)	202 (18.6)
AF, n (%)	375 (37.4)
Smoking, n (%)	
Never	523 (49.7)
Ex-smoker	416 (39.5)
Current	114 (10.8)
Pre-existing cognitive impairment, n (%)	217 (36.4)
Previous cerebral ischaemic event, n (%)	226 (21.8)
Previous ICH, n (%)	46 (4.3)
ApoE ε2, presence, n (%)	189 (20.7)
ApoE ε4, presence, n (%)	256 (28.1)
Medications	
Antiplatelet use prior to ICH, n (%)	267 (24.6)
Anticoagulant use prior to ICH, n (%)	436 (40.1)
Antiplatelet at discharge, n (%)	65 (6.4)
Anticoagulant at discharge, n (%)	113 (10.7)
Clinical features at study entry	
GCS, median (IQR)	15 (14 to 15)
NIHSS, median (IQR)	7 (3 to 13)
Imaging features at study entry	
Lacunes, presence, n (%)	98 (9.0)
Van Swieten Score (WMH), median (IQR)	0 (o to 2)
ICH location	
Lobar	447 (40.9)
Deep	546 (50.0)
Cerebellar	65 (6.0)
Brainstem	34 (3.1)

Table 3.1.2: Univariable analyses for recurrent ICH events

Competing risk analyses completed with occurrence of an ischaemic event (IE) or death as the competing risk.

	Cox regression			Competing risk regression (IE)			Competing risk regression (death)		
	HR	**95% CI**	**p value**	**SHR**	**95% CI**	**p value**	**SHR**	**95% CI**	**p value**
Age, per year increase	1.03	1.00 to 1.06	0.039	1.03	1.00 to 1.06	0.071	1.02	0.99 to 1.05	0.205
Sex, male	0.89	0.50 to 1.61	0.709	0.90	0.50 to 1.62	0.727	0.92	0.51 to 1.65	0.778
Hypertension	1.02	0.54 to 1.89	0.961	1.01	0.54 to 1.88	0.976	0.98	0.52 to 1.82	0.937
Hypercholesterolaemia	1.15	0.63 to 2.09	0.651	1.13	0.62 to 2.05	0.700	1.13	0.62 to 2.05	0.698
Diabetes mellitus	1.35	0.67 to 2.73	0.403	1.32	0.65 to 2.67	0.438	1.29	0.64 to 2.60	0.480
AF	0.91	0.47 to 1.74	0.767	0.87	0.45 to 1.66	0.668	0.76	0.40 to 1.46	0.411
Smoking	0.92	0.59 to 1.42	0.695	0.92	0.60 to 1.40	0.696	0.92	0.60 to 1.41	0.702
Pre-existing cognitive impairment	1.18	0.54 to 2.60	0.680	1.18	0.54 to 2.62	0.676	1.09	0.49 to 2.39	0.833
Previous cerebral ischaemic event	2.33	1.23 to 4.40	0.009	2.24	1.19 to 4.23	0.013	2.17	1.15 to 4.09	0.017
Previous ICH	5.00	2.22 to 11.24	<0.0001	5.00	2.24 to 11.16	<0.0001	4.65	2.08 to 10.41	<0.0001
APOE ε2, presence	1.84	0.93 to 3.65	0.080	1.82	0.92 to 3.60	0.086	1.81	0.92 to 3.59	0.088
APOE ε4, presence	1.00	0.50 to 2.02	0.997	0.99	0.49 to 1.99	0.978	1.05	0.52 to 2.11	0.892
Medications									
Antiplatelet use prior to ICH	2.24	1.24 to 4.04	0.008	2.23	1.23 to 4.04	0.008	2.29	1.27 to 4.14	0.006
Anticoagulant use prior to ICH	0.66	0.34 to 1.27	0.213	0.63	0.33 to 1.22	0.170	0.57	0.30 to 1.10	0.093
Antiplatelet at discharge	1.05	0.32 to 3.38	0.940	1.07	0.33 to 3.45	0.905	1.05	0.33 to 3.35	0.941
Anticoagulant at discharge	0.60	0.18 to 1.92	0.387	0.60	0.18 to 1.93	0.389	0.59	0.18 to 1.92	0.384
Imaging features at study entry									
Lacunes, presence	1.27	0.50 to 3.22	0.611	1.28	0.50 to 3.26	0.607	1.27	0.50 to 3.24	0.618
Van Swieten Score, per point increase	1.30	1.08 to 1.56	0.006	1.29	1.06 to 1.57	0.013	1.25	1.03 to 1.52	0.026
ICH location, lobar (vs non-lobar)	5.40	2.60 to 11.24	<0.0001	5.42	2.61 to 11.28	<0.0001	5.22	2.51 to 10.84	<0.0001

Figure 3.1.1: Unadjusted Kaplan-Meier failure estimates for recurrent ICH, comparing patients with lobar and non-lobar ICH

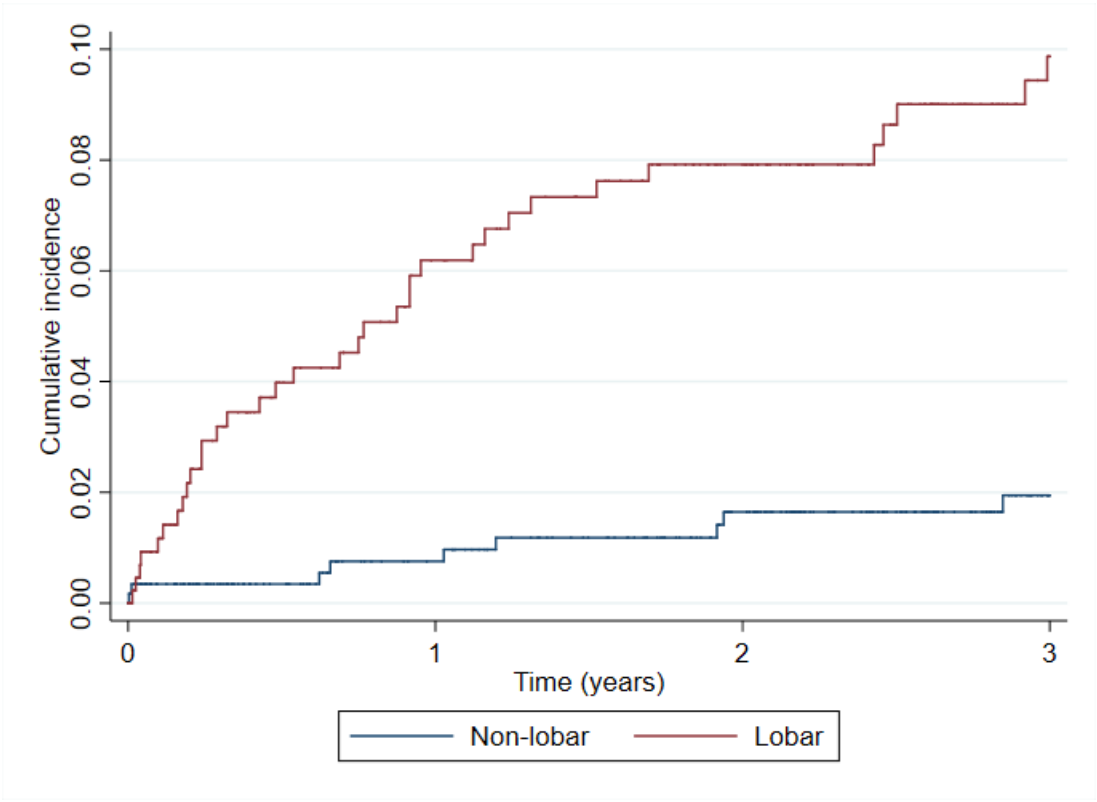


Figure 3.1.2: Unadjusted competing risk analyses for recurrent ICH, comparing patients with lobar and non-lobar ICH (occurrence of an ischaemic event as the competing risk)

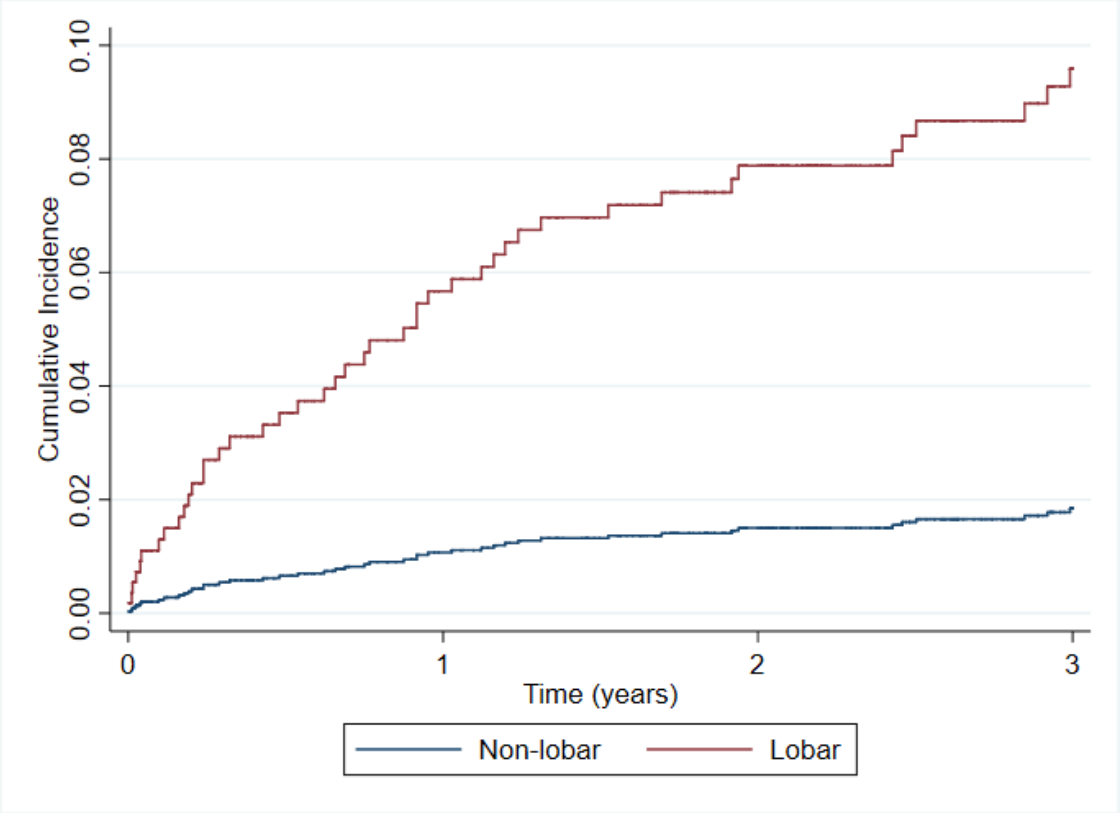


Figure 3.1.3: Unadjusted competing risk analyses for recurrent ICH, comparing patients with lobar and non-lobar ICH (occurrence of death as the competing risk)

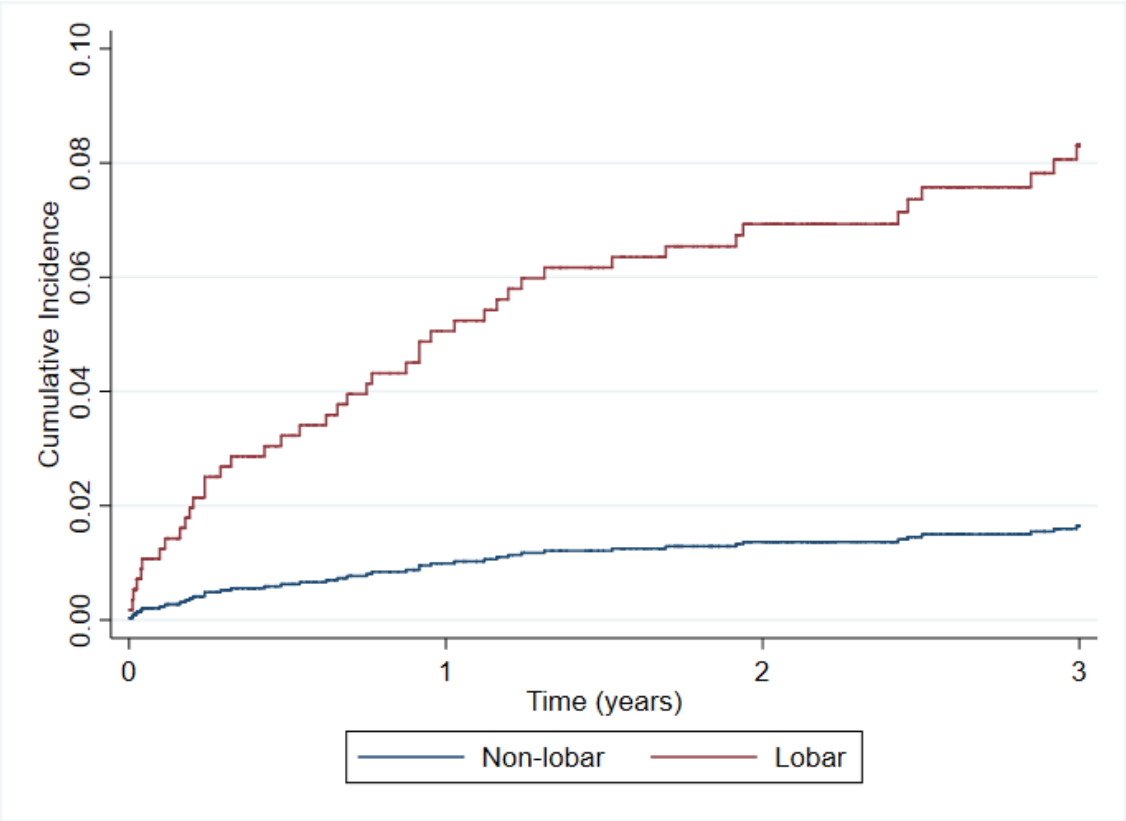


Table 3.1.3: Multivariable analyses for recurrent ICH events

Competing risk analyses completed with occurrence of an ischaemic event (IE) or death as the competing risk

	Cox regression			Competing risk regression (IE)			Competing risk regression (death)		
	HR	**95% CI**	**p value**	**SHR**	**95% CI**	**p value**	**SHR**	**95% CI**	**p value**
ICH location, lobar (vs non-lobar)	8.70	3.29 to 23.03	<0.0001	8.55	3.28 to 22.28	<0.0001	8.10	3.11 to 21.10	<0.0001
Age, per year increase	1.01	0.98 to 1.05	0.493	1.01	0.97 to 1.06	0.493	1.01	0.97 to 1.05	0.734
Previous cerebral ischaemic event	2.32	1.12 to 4.79	0.023	2.19	0.98 to 4.86	0.055	1.99	0.88 to 4.53	0.100
Previous ICH	3.87	1.16 to 12.93	0.028	3.70	1.05 to 12.99	0.042	2.46	0.62 to 9.69	0.198
APOE ε2	1.57	0.76 to 3.25	0.223	1.54	0.76 to 3.11	0.233	1.68	0.83 to 3.42	0.154
Antiplatelet use prior to ICH	2.62	1.31 to 5.24	0.006	2.55	1.25 to 5.19	0.010	2.68	1.31 to 5.47	0.007
Van Swieten Score, per point increase	1.27	1.01 to 1.59	0.041	1.26	1.00 to 1.59	0.051	1.17	0.93 to 1.48	0.186

Table 3.1.4: Univariable Cox regression analyses for subsequent cerebral ischaemic events

Competing risk analyses completed with occurrence of another ICH event or death as the competing risk.

	Cox regression			Competing risk regression (ICH)			Competing risk regression (death)		
	HR	**95% CI**	**p value**	**SHR**	**95% CI**	**p value**	**SHR**	**95% CI**	**p value**
Age, per year increase	1.03	1.01 to 1.05	0.004	1.03	1.01 to 1.05	0.004	1.02	1.00 to 1.04	0.052
Sex, male	0.80	0.50 to 1.28	0.347	0.81	0.50 to 1.29	0.367	0.82	0.52 to 1.32	0.416
Hypertension	0.91	0.56 to 1.49	0.710	0.91	0.56 to 1.49	0.707	0.87	0.53 to 1.42	0.570
Hypercholesterolaemia	1.69	1.05 to 2.73	0.031	1.69	1.05 to 2.72	0.032	1.65	1.02 to 2.66	0.040
Diabetes mellitus	1.36	0.77 to 2.42	0.288	1.34	0.75 to 2.39	0.318	1.28	0.72 to 2.28	0.397
AF	2.92	1.77 to 4.82	<0.0001	2.91	1.76 to 4.81	<0.0001	2.36	1.43 to 3.90	0.001
Smoking	0.96	0.67 to 1.36	0.804	0.96	0.67 to 1.38	0.841	0.96	0.67 to 1.39	0.837
Pre-existing cognitive impairment	1.01	0.49 to 2.10	0.972	1.01	0.49 to 2.11	0.973	0.91	0.44 to 1.89	0.801
Previous cerebral ischaemic event	2.95	1.80 to 4.83	<0.0001	2.87	1.75 to 4.70	<0.0001	2.70	1.65 to 4.41	<0.0001
Previous ICH	0.79	0.19 to 3.23	0.743	0.73	0.18 to 3.00	0.662	0.72	0.18 to 2.97	0.653
APOE ε2, presence	1.46	0.83 to 2.59	0.193	1.43	0.81 to 2.54	0.219	1.43	0.81 to 2.54	0.219
APOE ε4, presence	1.21	0.71 to 2.07	0.485	1.22	0.71 to 2.09	0.464	1.29	0.76 to 2.21	0.349
Medications									
Antiplatelet use prior to ICH	1.13	0.66 to 1.94	0.659	1.09	0.64 to 1.87	0.751	1.16	0.67 to 1.98	0.598
Anticoagulant use prior to ICH	2.56	1.58 to 4.15	<0.0001	2.58	1.59 to 4.16	<0.0001	2.13	1.32 to 3.44	0.002
Antiplatelet at discharge	0.22	0.03 to 1.62	0.139	0.23	0.03 to 1.62	0.139	0.22	0.03 to 1.61	0.137
Anticoagulant at discharge	0.98	0.45 to 2.15	0.961	0.99	0.45 to 2.16	0.978	0.97	0.44 to 2.11	0.937
Imaging features at study entry									
Lacunes, presence	0.79	0.32 to 1.96	0.608	0.77	0.31 to 1.92	0.578	0.78	0.31 to 1.95	0.597
Van Swieten Score, per point increase	1.25	1.07 to 1.46	0.004	1.23	1.06 to 1.43	0.006	1.19	1.02 to 1.39	0.024
ICH location, lobar (vs non-lobar)	1.04	0.64 to 1.69	0.856	0.99	0.61 to 1.60	0.970	1.01	0.62 to 1.63	0.982

Figure 3.1.4: Unadjusted Kaplan-Meier failure estimates for subsequent ischaemic events, comparing patients with lobar and non-lobar ICH

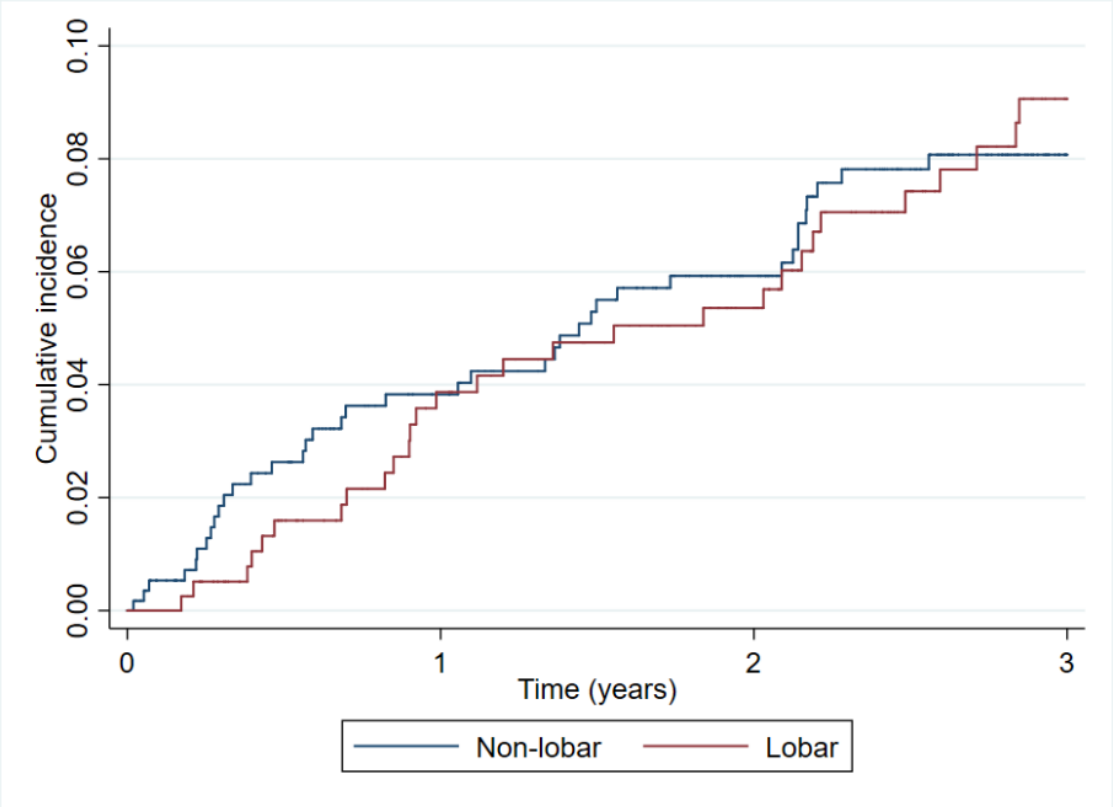


Figure 3.1.5: Unadjusted competing risk analyses for subsequent ischaemic events, comparing patients with lobar and non-lobar ICH (occurrence of recurrent ICH as the competing risk)

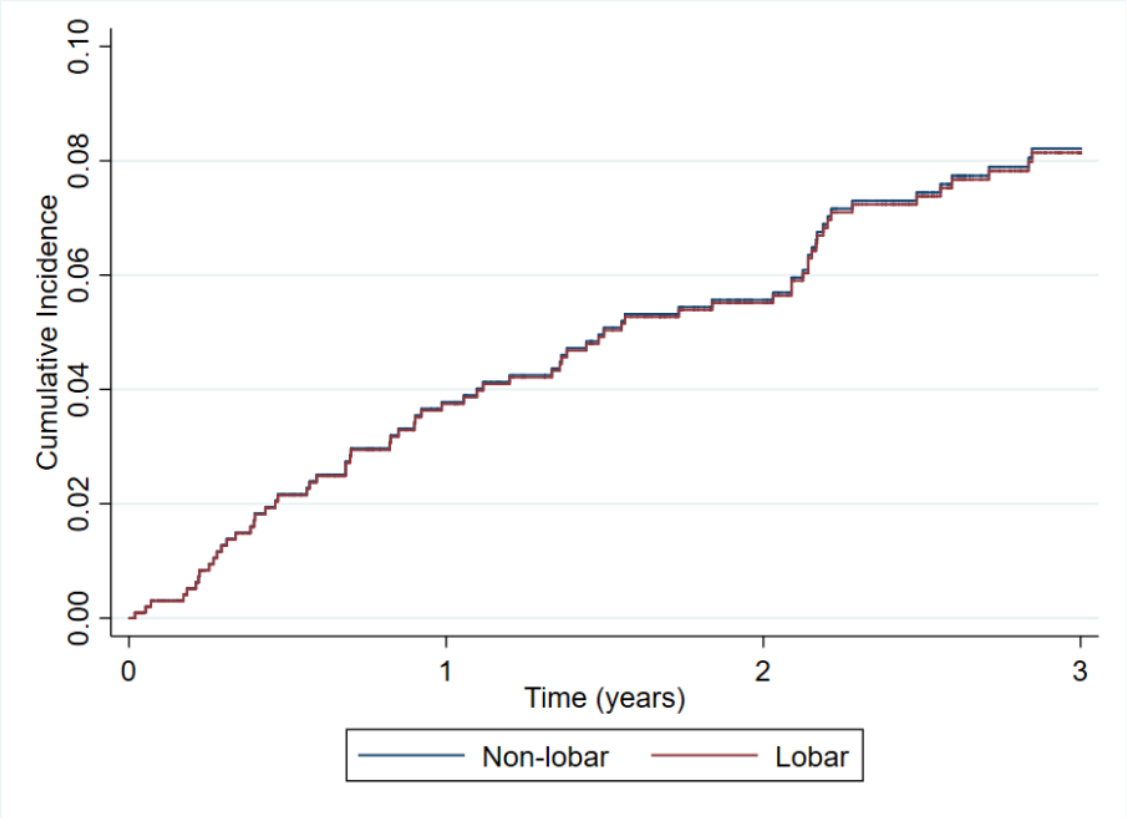


Figure 3.1.6: Unadjusted competing risk analyses for subsequent ischaemic events, comparing patients with lobar and non-lobar ICH, (occurrence of death as the competing risk)

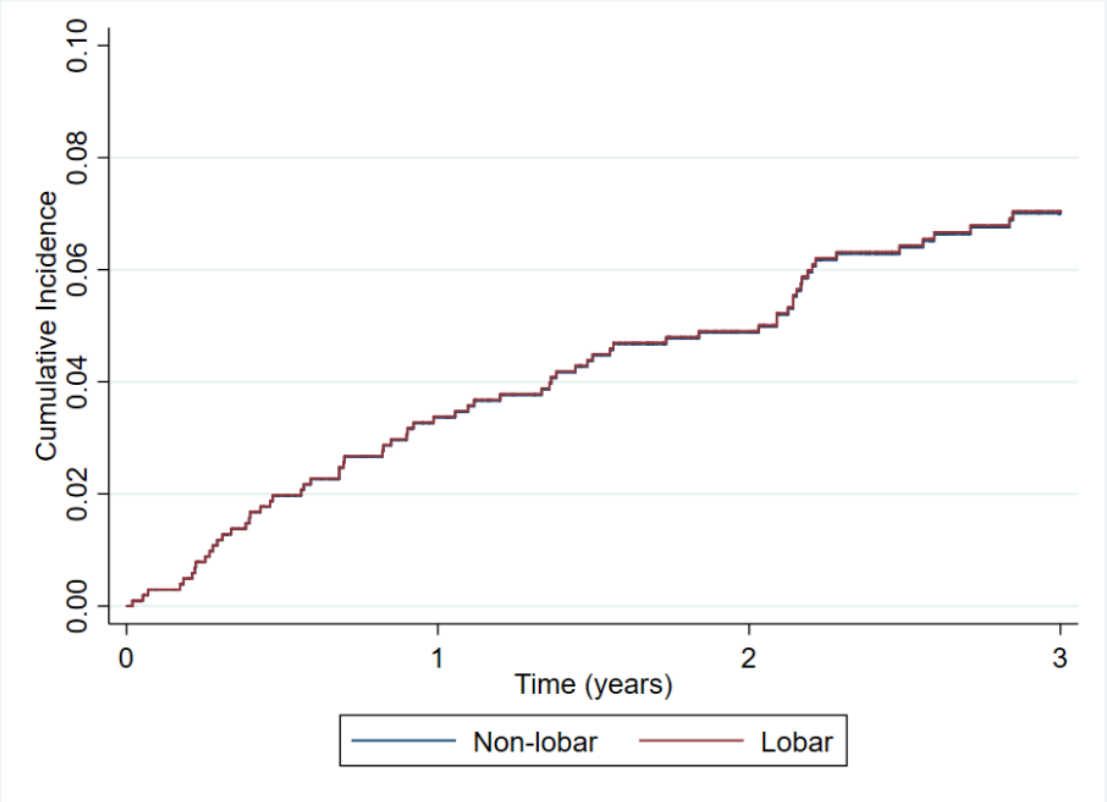


Table 3.1.5: Multivariable Cox regression for subsequent cerebral ischaemic events

Competing risk analyses completed with occurrence of another ICH event or death as the competing risk.

	Cox regression			Competing risk regression (ICH)			Competing risk regression (death)		
	HR	**95% CI**	**p value**	**SHR**	**95% CI**	**p value**	**SHR**	**95% CI**	**p value**
ICH location, lobar (vs non-lobar)	1.00	0.58 to 1.71	0.994	0.95	0.55 to 1.64	0.853	1.04	0.60 to 1.80	0.887
Age	1.03	1.00 to 1.06	0.053	1.03	1.00 to 1.06	0.058	1.01	0.99 to 1.04	0.281
Hypercholesterolaemia	1.24	0.71 to 2.16	0.448	1.24	0.70 to 2.21	0.457	1.30	0.74 to 2.31	0.365
AF	1.11	0.51 to 2.40	0.791	1.11	0.50 to 2.43	0.801	1.01	0.47 to 2.21	0.972
Previous cerebral ischaemic event	2.14	1.21 to 3.77	0.009	2.08	1.15 to 3.78	0.016	2.10	1.17 to 3.76	0.013
Anticoagulant use prior to ICH	1.89	0.90 to 4.10	0.107	1.96	0.88 to 4.37	0.102	1.84	0.83 to 4.09	0.136
Van Swieten Score, per point increase	1.12	0.93 to 1.34	0.251	1.11	0.93 to 1.33	0.252	1.11	0.92 to 1.34	0.264

3.1.4 Discussion

Our main findings are: (1) at 3 year follow up, there were fewer ICH events than cerebral ischaemic events (45 vs 70); (2) there was a difference in absolute event rates for recurrent ICH events for patients with lobar and non-lobar ICH (3.77 vs 0.69 per 100 patient-years), and lobar ICH location was independently associated with a higher risk of recurrent ICH events; and (3) absolute event rates for subsequent ischaemic events were similar for lobar and non-lobar groups (3.12 vs 2.97 per 100 patient-years), and there was no association between ICH location and the risk of subsequent cerebral ischaemic events. In addition to ICH location, recurrent ICH events were associated with a history of previous ischaemic events, antiplatelet use prior to study entry, and increasing Van Swieten score, whereas cerebral ischaemic events were associated with increasing age, AF, and a history of previous ischaemic events.

Our results provide new information that could guide the complex clinical decision-making processes associated with relative haemorrhagic and ischaemic risk in these patients. Whilst variables known to be associated with increased ischaemic stroke risk (increasing age, AF, previous cerebral ischaemic events) were associated with subsequent cerebral ischaemic events, measures that might reflect small vessel disease (such as Van Swieten score) did not show any independent association. Additionally, whilst one might hypothesise that ICH location might predict the occurrence of later cerebral ischaemic events (given the hypothesised increase in ischaemic risk with DPA), we did not find this to be the case; this could either be interpreted as showing that the ischaemic risk of deep perforator arteriopathy may be overestimated, or that the ischaemic risk in those with lobar ICH (and presumably CAA) is underestimated. It is well recognised that lobar haemorrhage is not only due to CAA (one recent study found that of 62 patients with lobar ICH, 26 had absent or

mild CAA (58)), and additionally there is significant pathological overlap between these two small vessel diseases (the same study found that of 36 patients with moderate or severe CAA, 26 also had evidence of another small vessel disease (58)); further work is needed to clarify the complex interactions between these factors.

The finding that lobar ICH is associated with a higher recurrence rate is in keeping with previous work (2, 20, 22); this is believed to reflect the association of lobar ICH with CAA. However, the frequent co-existence of CAA with other small vessel pathologies in patients with lobar ICH might suggest that lobar ICH occurs in the context of "severe" small vessel disease – be it CAA, or DPA, or both. We also found an association with a prior history of ICH was associated with subsequent ICH occurrence, suggesting that some individuals are particularly "bleeding-prone", independent of ICH location. The observed association with previous cerebral ischaemic events might reflect that these events (in particular lacunar infarction) are associated with more severe small vessel disease (specifically, DPA); the association with prior antiplatelet use might be a surrogate marker for this (although other explanations are possible; this observation could also reflect that those taking antiplatelet medications prior to ICH are more likely to be restarted on them, following discharge). Taken together, this suggests that factors beyond ICH location are important for identifying those at highest risk of recurrent ICH, and that severe small vessel disease, regardless of subtype, may be important.

The strengths of this study are its large size, the study design (in which data was collected prospectively, from multiple centres) and the detailed clinical and imaging data available for participants. Limitations include those inherent to the coding of hospital episodes (with regard to accuracy) and the lack of central adjudication of events. This method of ascertainment may also result in some events being missed, for example if patients were treated in non-NHS facilities (such as those outside the

UK or private hospitals), or in the case of minor events, which might not have resulted in a hospital attendance. Additionally, only the first cerebrovascular event was considered; this work did not explore repeated events, and in patients who had both ischaemic and haemorrhagic events, only the first event was included. The number of outcome events was relatively low, and as a consequence we were unable to explore the role of ICH location in further detail (i.e. in those with cerebellar or brainstem ICH). MR data was not available for all patients, and as a consequence we were unable to provide more detailed information on the nature and severity of any underlying cerebral small vessel disease. We also acknowledge that our results might be subject to selection bias, as our cohort only included ICH survivors. Finally, as noted above, we did not have information on the prescription of antiplatelet or anticoagulant medications following discharge, and this could have influenced our results.

In conclusion, we report fewer ICH events than cerebral ischaemic events at 3 years in ICH survivors; it is possible that the ischaemic risk in those presenting with ICH is underestimated. Lobar ICH location is associated with a higher risk of recurrent ICH events than deep ICH, and with features that may reflect small vessel disease severity. Outstanding questions remain about whether these associations with lobar ICH occur reflect a single small vessel pathology or the severity of small vessel disease more generally; further work is needed to disentangle the complex interaction between these small vessel diseases, and their impact on an individual's future stroke risk.

3.2 Long-term mortality following intracerebral haemorrhage and role of time-varying effects

3.2.1 Introduction

As has been described, most research on outcomes following ICH has focussed on short term mortality, reflecting the high rates of early death associated with this stroke subtype (20, 309). Many of the factors associated with this early mortality relate to ICH severity, for example neurological examination findings on admission (using scores like the Glasgow Coma Scale, GCS, or National Institutes of Health Stroke Scale, NIHSS), haemorrhage volume, infratentorial location, and the presence of intraventricular extension (317), and this is reflected in the many prognostic scores which aim to predict outcome in the short term (318-325). Additionally, patients with ICH are more likely to receive palliative care on the first day of their admission than patients with ischaemic strokes of similar severity (326); these and other "early care limitations" are associated with mortality in both the short and long-term (327).

In recent years, perceptions of ICH are changing. Acute interventions including aggressive blood pressure management, the reversal of anticoagulation, and prompt neurosurgical referral might improve prognosis in patients with ICH (310). There are also new pharmacological strategies being tested in ICH, for example tranexamic acid, which might further improve prognosis in the short term (311). This "active" immediate management of ICH is focussed on treating factors associated with early mortality; if these interventions are successful and there are consequently more ICH survivors, a better understanding of the characteristics that influence subsequent mortality will be crucial. Moreover, an improved understanding of the factors that influence "late" death following ICH might identify potentially modifiable risk factors that could improve long-term outcomes for these patients (309).

We aimed to address these questions using data from the prospective CROMIS-2 ICH study. Our aims were: (1) to describe the frequency of mortality events up to 3 years following ICH, and the factors associated with mortality over this time period (including those relating to SVD); and (2) to evaluate whether the effect of these factors vary with time. We hypothesised that factors relating to the severity of the acute ICH would not be associated with death at later (beyond 6 months) time points.

3.2.2 Methods

3.2.2.1 Participants

We included patients recruited to the CROMIS-2 ICH study, details for which have been described earlier in this thesis (Section 2.5.2.1).

Patients were considered to have pre-existing cognitive impairment if they had a formal diagnosis of dementia or cognitive impairment at study entry, or if they scored more than 3.3 on the 16-item IQCODE, as previously described in this thesis (Section 2.5.2.1). *APOE* genotype was established from peripheral blood samples (by Isabel Hostettler, Clinical Research Associate); the method for this has been previously described (313).

3.2.2.2 Outcomes

The outcome of interest for this project was death within 3 years of study entry. As described earlier in this thesis (Section 3.1.2.2), outcomes for the first 6 months following the index event were collected using multiple ascertainment methods, and outcome data from 6 months to 3 years were compiled from notifications from NHS Digital. Patients were censored at the date of death; if they did not die, they were censored either 3 years following the ICH that resulted in study entry, or at the time

of the study's last notification of deaths from NHS Digital (31/10/2017), with the earlier date being used in these cases.

3.2.2.3 Imaging

Brain CT imaging was acquired acutely at the time of the index event as part of the patient's routine clinical care. Imaging analysis was carried out by a clinical research associate (Duncan Wilson) and an MSc student (Surabhika Lunawat), both of whom were trained in neuroimaging rating and blinded to the participant clinical details. Haematoma location, lacunes, and WMH were rated by Duncan Wilson, as described earlier in this thesis (Section 3.1.2.3). Haematoma volume was rated (Surabhika Lunawat) using a semi-automated planimetric method, which has previously been described (231, 328). Intraventricular (IV) extension was defined as the presence of any blood within the cerebral ventricular system (Duncan Wilson).

3.2.2.4 Statistics

Statistical analysis was performed by the candidate using Stata (Version 15.1). Univariable Cox regression was used to compare clinical and imaging variables associated with death; this was used due to the variation in length of follow up for each patient. Multivariable Cox regression analysis was then performed; adjustments were made for all variables with $p<0.10$ in univariable analyses. The proportional-hazards assumption test based on Schoenfeld residuals was applied to all Cox models (univariable and multivariable). Weibull regression analyses with and without individual frailty terms were performed, in order to quantify unobserved heterogeneity.

In order to test our hypothesis that the variables influencing death vary with time, we initially dichotomised time following ICH into "early" (before 6 months) and "late" (after 6 months) periods and used univariable Cox regression to calculate hazard ratios in

these two periods. Variables where the 95% confidence intervals did not cross 1 were considered as statistically significant. In order to further explore these time-varying effects, the time-varying effect of each variable was allowed to vary linearly with time. Likelihood ratio tests (LRT) were used to evaluate the difference between the constant hazard ratio (i.e. at time 0, study entry) and the time-varying hazard ratio; values <0.05 were considered as significant (this is equivalent to violating the proportional-hazards assumption, which assumes that that the hazard ratio is constant over time). For those variables with a significant time-varying effect, we then calculated hazard ratios at 1 year intervals i.e. at study entry (time 0), and then 1 year, 2 years and 3 years, in order to assess how the hazard ratio varies with time.

3.2.3 Results

All 1094 patients recruited to CROMIS-2 ICH were included (Table 3.2.1). Follow up was for a total 2613.48 patient-years (median 3.00 years, IQR 2.31 to 3.00 years). There were 306 deaths (absolute event rate 117.1 per 1000 patient-years, 95% CI 104.7 to 131.0 per 1000 patient-years). Figure 3.2.1 shows unadjusted Kaplan-Meier estimates for death.

In univariable Cox regression analyses (Table 3.2.2), death was associated with age at study entry, sex, hypercholesterolemia, diabetes mellitus, AF, smoking at time of ICH, pre-existing cognitive impairment, history of previous cerebral ischaemic events, pre-event mRS, anticoagulant use prior to ICH, GCS and NIHSS at admission. There were also associations with white matter disease severity (as measured by the Van Swieten score), higher ICH volumes, and the presence of intraventricular extension.

A multivariable Cox regression model including these variables in addition to hypertension, history of ICH prior to the index event and *APOE* ε4 genotype (Table

3.2.3) found associations with age at study entry (per year increase, HR 1.12, 95% CI 1.08 to 1.17, p<0.0001), smoking (HR 3.45, 95% CI 1.12 to 10.60, p=0.031), pre-event mRS (per point increase, HR 1.31, 95% CI 1.02 to 1.68, p=0.036) and NIHSS at presentation (per point increase, HR 1.10, 95% CI 1.04 to 1.17, p=0.001). Weibull regression (adjusted for the same variables) found similar values for the HRs; addition of an individual frailty term resulted in a statistically significant frailty parameter (θ 1.27, p=0.035), suggesting that there is unobserved heterogeneity contributing to the outcome.

3.2.3.1 Associations of "early" vs "late" death

Of the 306 death events, 156 occurred within 6 months of the index haemorrhage event ("early"), and 150 deaths occurring after 6 months and within 3 years of the index ICH ("late"). The baseline characteristics for both groups are shown in Table 3.2.1.

Early death (Table 3.2.4) was associated with age at study entry, hypertension, diabetes mellitus, AF, a history of previous cerebral ischaemic events, pre-event mRS, anticoagulant use prior to ICH, GCS, and NIHSS. Imaging features at study entry that were significantly associated with early death were Van Swieten score, ICH volume, and the presence of intraventricular extension. In a multivariable model including these variables, age at study entry (per year increase, HR 1.06, 95% CI 1.02 to 1.09, p=0.001), hypertension (HR 2.44, 95% CI 1.25 to 4.77, p=0.009), pre-event mRS (per point increase, HR 1.32, 95% CI 1.08 to 1.60, p=0.006), admission NIHSS (per point increase, HR 1.12, 95% CI 1.07 to 1.17, p<0.0001), and ICH volume > 60ml (HR 3.55, 95% CI 1.54 to 8.17, p=0.003) remained associated with early death.

When considering late death events (Table 3.2.4), age, AF, smoking, pre-event cognitive impairment, previous cerebral ischaemic event, anticoagulant use prior to index ICH, pre-event mRS, increasing van Swieten score and the presence of intraventricular extension showed significant associations. In a multivariable model including all variables with a significant association with late death, only age at study entry (per year increase, HR 1.05, 95% CI 1.01 to 1.08, p=0.005), pre-event mRS (per point increase, HR 1.45, 95% CI 1.16 to 1.82, p=0.001), anticoagulant use prior to ICH (HR 2.37, 95% CI 1.14 to 4.93, p=0.021) and the presence of intraventricular extension (HR 1.95, 95% CI 1.14 to 3.33, p=0.015) remained associated with late death.

We then investigated which baseline characteristics showed a significant change in HR between the early and the late periods (Table 3.2.4). We found that HRs for the presence of *APOE* ε2 (early HR 1.40, 95% CI 0.93 to 2.11, vs late HR 0.70, 95% CI 0.44 to 1.13, p=0.032), GCS (per point increase, early HR 0.80, 95% CI 0.76 to 0.84, vs late HR, 0.93, 95% CI 0.86 to 1.00, p=0.001), NIHSS (per point increase, early HR 1.11, 95% CI 1.08 to 1.14, vs late HR 1.00, 95% CI 0.97 to 1.04, p<0.0001), and ICH volume > 60ml (early HR 4.85, 95% CI 3.01 to 7.83, vs late HR 1.40, 95% CI 0.62 to 3.18, p=0.010) showed evidence of significant change between the early and late periods.

3.2.3.2 Further exploratory analysis of time-varying effects

Variables which showed significant linear time-varying effects were history of a previous cerebral ischaemic event (p=0.0261), admission GCS (p=0.0108), NIHSS (p<0.00001), Van Swieten score (p=0.0349), and ICH volume (p=0.0439). These models were then used derive HR for each variable at study entry, and then 1 year,

2 years and 3 years following this; the time-varying effects of these variables are shown in Table 3.2.5. The hazard ratios of previous cerebral ischaemic events and Van Swieten score increased with time, whilst those for NIHSS and ICH volume decreased with time. The protective (negative association) of GCS also decreased with time.

Table 3.2.1: Baseline characteristics

Percentage values were calculated using the total number of patients for whom data was available as the denominator.

		All	Alive	Early death (<6 months)	Late death (≥6 months)
n		1094	788 (72.0)	156 (14.3)	150 (13.7)
Age, years, mean (SD)		73.3 (12.5)	70.3 (12.4)	81.1 (9.4)	80.7 (8.5)
Sex, male, n (%)		628 (57.4)	468 (59.4)	78 (50.0)	82 (54.7)
Hypertension, n (%)		718 (66.7)	505 (65.3)	114 (73.6)	99 (66.9)
Hypercholesterolaemia, n (%)		467 (44.0)	322 (42.0)	71 (47.7)	74 (50.3)
Diabetes mellitus, n (%)		202 (18.6)	132 (16.9)	38 (24.4)	32 (21.6)
AF, n (%)		375 (37.4)	215 (30.1)	81 (56.3)	79 (55.2)
Smoking (at time of ICH), n (%)		114 (10.8)	94 (12.4)	12 (8.0)	8 (5.6)
Pre-existing cognitive impairment, n (%)		217 (36.4)	135 (32.0)	41 (43.6)	41 (51.3)
Previous cerebral ischaemic event, n (%)		226 (21.8)	140 (18.5)	41 (28.7)	45 (32.4)
Previous ICH, n (%)		46 (4.3)	28 (3.6)	10 (6.7)	8 (5.6)
Pre-event mRS, median (IQR)		0 (0 to 1)	0 (0 to 1)	1 (0 to 3)	1 (0 to 2)
APOE ε2, presence, n (%)		189 (20.7)	138 (20.8)	31 (26.5)	20 (15.3)
APOE ε4, presence, n (%)		256 (28.1)	196 (29.5)	24 (20.5)	36 (27.5)
Medications					
Antiplatelet use prior to ICH, n (%)		267 (24.6)	193 (24.7)	38 (24.5)	36 (24.2)
Anticoagulant use prior to ICH, n (%)		436 (40.1)	261 (33.4)	86 (55.5)	89 (59.3)
Antiplatelet at discharge, n (%)		65 (6.4)	46 (6.2)	8 (6.2)	11 (7.8)
Anticoagulant at discharge, n (%)		113 (10.7)	78 (10.2)	14 (9.5)	21 (14.5)
Clinical features at study entry					
GCS, median (IQR)		15 (14 to 15)	15 (14 to 15)	14 (11 to 15)	15 (14 to 15)
NIHSS, median (IQR)		7 (3 to 13)	6 (3 to 11)	14 (7 to 19)	6 (3 to 12)
Imaging features at study entry					
Lacunes, presence, n (%)		98 (9.0)	69 (8.8)	15 (9.6)	14 (9.3)
Van Swieten Score (WMH), median (IQR)		0 (o to 2)	0 (0 to 2)	1 (0 to 3)	2 (0 to 3)
ICH location	Infratentorial	99 (9.1)	69 (8.8)	12 (7.7)	18 (12.0)
	Deep	546 (50.0)	398 (50.6)	69 (44.2)	79 (52.7)
	Lobar	447 (40.9)	319 (40.6)	75 (48.1)	53 (35.3)
ICH volume	<30ml	886 (85.9)	655 (89.0)	106 (70.7)	125 (85.6)
	30 – 60ml	99 (9.6)	60 (8.2)	24 (16.0)	15 (10.3)
	<60ml	47 (4.6)	21 (2.9)	20 (13.3)	6 (4.1)
IV extension		301 (27.7)	183 (23.4)	68 (43.6)	50 (33.6)

Table 3.2.2: Univariable Cox regression analyses for death

†indicates variables that violated the proportional-hazards assumption.

		HR	95% CI	p value
Age, per year increase		1.08	1.07 to 1.10	<0.0001
Sex, male		0.79	0.63 to 0.99	0.037
Hypertension		1.24	0.97 to 1.60	0.087
Hypercholesterolaemia		1.26	1.00 to 1.58	0.050
Diabetes mellitus		1.38	1.05 to 1.80	0.019
AF		2.48	1.97 to 3.14	<0.0001
Smoking, current†		0.57	0.36 to 0.90	0.016
Pre-existing cognitive impairment		1.67	1.24 to 2.25	0.001
Previous cerebral ischaemic event†		1.68	1.30 to 2.16	<0.0001
Previous ICH		1.57	0.98 to 2.54	0.062
Pre-event mRS, per point increase		1.53	1.41 to 1.66	<0.0001
APOE ε2, presence		1.01	0.74 to 1.37	0.956
APOE ε4, presence		0.78	0.58 to 1.04	0.097
Medications				
Antiplatelet use prior to ICH		0.97	0.75 to 1.27	0.847
Anticoagulant use prior to ICH		2.31	1.84 to 2.90	<0.0001
Antiplatelet at discharge		1.09	0.69 to 1.74	0.708
Anticoagulant at discharge		1.15	0.80 to 1.63	0.450
Clinical features at study entry				
GCS, per point increase†		0.85	0.81 to 0.88	<0.0001
NIHSS, per point increase†		1.06	1.04 to 1.09	<0.0001
Imaging features at study entry				
Lacunes, presence		1.05	0.72 to 1.55	0.785
Van Swieten Score (WMH), per point increase†		1.31	1.22 to 1.41	<0.0001
ICH location	Infratentorial	*Reference group*		0.7304
	Deep	0.89	0.60 to 1.32	
	Lobar	0.97	0.65 to 1.44	
ICH volume	<30ml	*Reference group*		<0.00001
	30 – 60ml	1.72	1.23 to 2.42	
	>60ml	3.08	2.05 to 4.62	
IV extension		1.87	1.48 to 2.35	<0.0001

Figure 3.2.1: Unadjusted Kaplan-Meier failure estimates for death

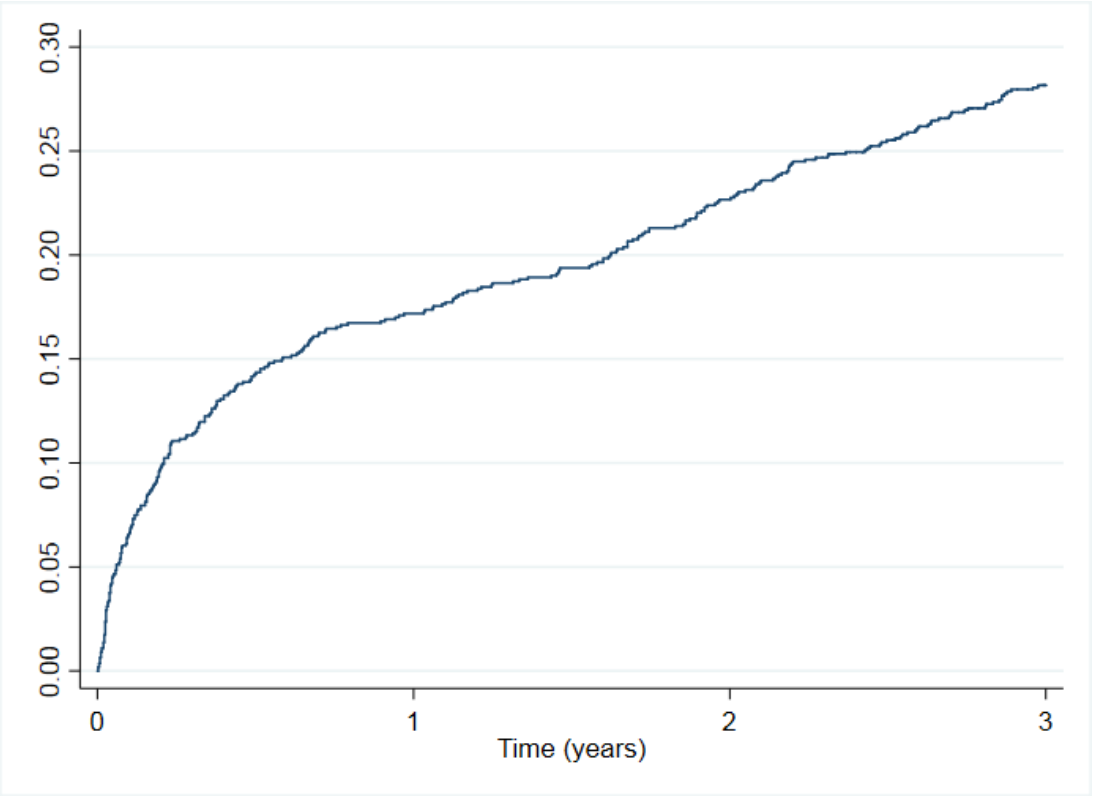


Table 3.2.3: Multivariable Cox regression model for predictors of death

		HR	95% CI	p value
Age, per year increase		1.12	1.08 to 1.17	<0.0001
Sex, male		1.49	0.82to 2.71	0.195
Hypertension		1.13	0.55 to 2.29	0.741
Hypercholesterolaemia		0.56	0.30 to 1.06	0.077
Diabetes mellitus		1.06	0.49 to 2.28	0.883
AF		0.78	0.31 to 1.97	0.600
Smoking, current		3.45	1.12 to 10.60	0.031
Pre-existing cognitive impairment		1.01	0.51 to 2.02	0.976
Previous cerebral ischaemic event		0.97	0.50 to 1.91	0.939
Previous ICH		4.44	0.93 to 21.25	0.061
Pre-event mRS, per point increase		1.31	1.02 to 1.68	0.036
APOE ε4, presence		0.58	0.29 to 1.18	0.135
Anticoagulant use prior to ICH		2.19	0.83 to 5.72	0.111
GCS, per point increase		1.07	0.84 to 1.36	0.586
NIHSS, per point increase		1.10	1.04 to 1.17	0.001
Van Swieten Score (WMH), per point increase		1.15	0.94 to 1.40	0.361
ICH volume	<30ml	*Reference group*		0.5082
	30 – 60ml	1.54	0.61 to 3.92	
	>60ml	0.65	0.18 to 2.2	
IV extension		1.34	0.70 to 2.58	0.381

Table 3.2.4: Univariable Cox regression analysis of time-varying effects during the early (before 6 months) and late (after 6 months) periods following ICH

Univariable hazard ratios for each characteristic obtained by fitting Cox regression models with time-varying effects (before/after 6 months). The time-varying coefficient p value compares the difference between the early and the late hazard ratios.

		"Early" , HR (95% CI)	**"Late", HR (95% CI)**	**Time-varying coefficient, p value**
Age, per year increase		1.08 (1.06 to 1.10)	1.10 (1.07 to 1.11)	0.360
Sex, male		0.73 (0.53 to 1.00)	0.85 (0.62 to 1.18)	0.500
Hypertension		1.43 (1.00 to 2.04)	1.08 (0.77 to 1.52)	0.270
Hypercholesterolaemia		1.18 (0.86 to 1.63)	1.34 (0.97 to 1.85)	0.585
Diabetes mellitus		1.44 (1.00 to 2.07)	1.31 (0.89 to 1.94)	0.729
AF		2.31 (1.66 to 3.21)	2.67 (1.92 to 3.71)	0.548
Smoking, current		0.70 (0.39 to 1.27)	0.45 (0.22 to 0.91)	0.337
Pre-existing cognitive impairment		1.39 (0.92 to 2.09)	2.07 (1.34 to 3.21)	0.191
Previous cerebral ischaemic event		1.49 (1.03 to 2.14)	1.90 (1.33 to 2.71)	0.346
Previous ICH		1.65 (0.87 to 3.14)	1.49 (0.73 to 3.04)	0.833
Pre-event mRS, per point increase		1.56 (1.40 to 1.74)	1.50 (1.33 to 1.69)	0.610
APOE ε2, presence		1.40 (0.93 to 2.11)	0.70 (0.44 to 1.13)	0.032
APOE ε4, presence		0.65 (0.42 to 1.02)	0.90 (0.61 to 1.32)	0.280
Medications				
Antiplatelet use prior to ICH		0.98 (0.68 to 1.42)	0.96 (0.66 to 1.40)	0.936
Anticoagulant use prior to ICH		1.97 (1.44 to 2.71)	2.73 (1.97 to 3.78)	0.164
Antiplatelet at discharge		0.95 (0.46 to 1.93)	1.23 (0.67 to 2.28)	0.582
Anticoagulant at discharge		0.85 (0.49 to 1.48)	1.48 (0.93 to 2.35)	0.134
Clinical features at study entry				
GCS, per point increase		0.80 (0.76 to 0.84)	0.93 (0.86 to 1.00)	0.001
NIHSS, per point increase		1.11 (1.08 to 1.14)	1.00 (0.97 to 1.04)	<0.0001
Imaging features at study entry				
Lacunes, presence		1.05 (0.62 to 1.79)	1.06 (0.61 to 1.84)	0.976
Van Swieten Score (WMH), per point increase		1.24 (1.12 to 1.37)	1.40 (1.26 to 1.55)	0.112
ICH location	Infratentorial	*Reference group*		
	Deep	1.04 (0.57 to 1.93)	0.79 (0.47 to 1.32)	0.494
	Lobar	1.42 (0.77 to 2.62)	0.67 (0.39 to 1.14)	0.067
ICH volume	<30ml	*Reference group*		
	30 – 60ml	2.20 (1.41 to 3.42)	1.29 (0.75 to 2.20)	0.131
	>60ml	4.85 (3.01 to 7.83)	1.40 (0.62 to 3.18)	0.010
IV extension		2.20 (1.61 to 3.02)	1.55 (1.11 to 2.18)	0.141

Table 3.2.5: Hazard ratios for variables with a significant time-varying effect, at time 0 (study entry), and then 1 year, 2 years and 3 years subsequently

Univariable hazard ratios evaluated at various times for each characteristic obtained by fitting Cox regression models with linear time-varying effects.

		Study entry (time 0), HR (95% CI)	1 year after ICH, HR (95% CI)	2 years after ICH, HR (95% CI)	3 years after ICH, HR (95% CI)
Previous cerebral ischaemic event		1.26 (0.87 to 1.82)	1.70 (1.32 to 2.20)	2.30 (1.59 to 2.32)	3.11 (1.73 to 5.60)
GCS, per point increase		0.81 (0.77 to 0.85)	0.86 (0.82 to 0.90)	0.91 (0.84 to 0.99)	0.97 (0.86 to 1.10)
NIHSS, per point increase		1.11 (1.08 to 1.14)	1.05 (1.03 to 1.08)	1.00 (0.96 to 1.04)	0.95 (0.89 to 1.01)
Van Swieten Score (WMH), per point increase		1.22 (1.10 to 1.35)	1.32 (1.22 to 1.42)	1.44 (1.29 to 1.61)	1.57 (1.31 to 1.87)
ICH volume	<30ml	*Reference group*	*Reference group*	*Reference group*	*Reference group*
	30 – 60ml	2.14 (1.35 to 3.38)	1.66 (1.16 to 2.36)	1.28 (0.71 to 2.32)	0.99 (0.39 to 2.56)
	>60ml	4.69 (2.80 to 7.84)	2.59 (1.59 to 4.20)	1.43 (0.57 to 3.59)	0.79 (0.18 to 3.38)

3.2.4 Discussion

Our main results are: (1) 72% of our cohort were alive at 3 years; death following ICH was independently associated with age at study entry, smoking, pre-event mRS and NIHSS at presentation, (2) “early” death (within 6 months of ICH) was associated with different factors when compared with “late” death in adjusted analyses, and when comparing these two periods, the presence of *APOE* ε2, GCS, NIHSS and ICH volume showed evidence of change over time (3) in further exploratory analyses where the time-varying effect of each variable was allowed to vary linearly with time, a history of a previous cerebral ischaemic event, GCS, NIHSS, Van Swieten score and ICH volume had significant effects, with the hazard ratios of previous cerebral ischaemic events and Van Swieten score increasing with time, whilst those for GCS, NIHSS and ICH volume decreased with time. Together, these results demonstrate that the factors associated with death following ICH in the longer-term are different to those associated with early mortality, and that the impact of some characteristics present at study entry vary with time.

Although some previous studies have considered longer term mortality (20, 309, 329-335), little is known about the factors that might influence this; associations with increasing age, diabetes mellitus, anticoagulant use prior to ICH and severe white matter disease (leukoaraiosis) have been reported (332, 333). In our study, we found that the only independent factors associated with mortality in the 3 years following ICH were age at study entry, smoking, pre-event mRS and NIHSS at presentation. However, we also found that different factors were associated with “early” (within 6 months) and “late” death. The factors that we found were independently associated with early death (age at study entry, hypertension, pre-event mRS, admission NIHSS, ICH volume > 60ml) are in keeping with other studies, and reflected in pre-existing prognostic scores which include these and other variables (318-325). We found that late death was associated with age at study entry, pre-event mRS, anticoagulant use prior to ICH and the presence

of intraventricular extension. Differences between our results and those previously reported are likely to reflect our method of considering early and late death independently; we observed similar results in this study in the associations observed "overall" (when considering all death events together). Additionally, we observed that four variables (*APOE* ε2, GCS, NIHSS and ICH volume > 60ml) showed significant differences in the magnitude of their effect before and after 6 months (although the hazard ratios for *APOE* ε2 were not statistically significant in themselves). This result confirms that whilst GCS, NIHSS and ICH volume are important predictors of early mortality, their effect changes significantly between the early and late periods, and thus they are less useful for predicting mortality in the longer-term, as we hypothesised.

Our analyses of linear time-varying effects on long-term mortality following ICH are novel and demonstrate the potentially complex interactions that can occur over time. In our exploratory analyses, we found that the hazard ratios for variables associated with initial stroke severity (GCS, NIHSS, ICH volume) were initially of high magnitude, but then decreased with time (in keeping with our dichotomised early versus late analyses). We additionally found that two variables, history of previous ischaemic events and Van Swieten score, show increasing hazard ratios with time, although these results should be interpreted cautiously as they are from unadjusted analyses (with age being one obvious potential confounder). These analyses also highlight the difficulties in defining what is "early" death, or a "short-term" outcome; further work that considers time-varying effects on mortality across longer time scales is needed to guide this.

Our study has a number of strengths, including the number of patients, its multicentre design, robust ascertainment of follow up events and the detailed clinical and radiological data available for each participant; however, we must also acknowledge some limitations of our work. Firstly, our cohort is arguably a survivor cohort with milder strokes (as reflected by the median NIHSS of 7, and the relatively low mortality rate), and thus our data might not be representative of those presenting with more severe haemorrhages.

We were unable to include details relating to the acute complications of ICH (such as haematoma expansion or obstructive hydrocephalus), or details relating to immediate care, either active (for example, aggressive blood pressure management, anticoagulation reversal, neurosurgical intervention (310)) or care-limiting (do not resuscitate orders or palliative pathways), all of which would impact mortality. Additionally, we were unable to comment on cause of death in our patients; the factors associated with “stroke-related” death may differ to those associated with death due to other causes. Finally, whilst we considered the time-varying effects of variables recorded at study entry, the status of these may have changed after this time-point (for example, smoking status, antiplatelet or anticoagulant use) and this could have influenced our results. The significance of the frailty term in our Weibull analysis suggests that there is unobserved heterogeneity contributing to mortality in our cohort, implying that there are factors which we either did not consider (which may include other life-limiting illnesses, for example chronic respiratory or renal diseases) or were unable to quantify fully.

We conclude that the factors associated with three-year mortality after ICH frequently relate to baseline patient characteristics, specifically age and premorbid functional status, as well as those relating ICH severity; however, the factors that influence mortality vary with time, with different variables are associated with early and late death. Further work evaluating these time-varying effects on longer term outcomes after ICH is needed.

4 BOCAA: Biomarkers and Outcomes in Cerebral Amyloid Angiopathy

4.1 Introduction

As discussed in the introduction to this thesis, whilst our ability to diagnose CAA has improved substantially in recent years, the vast majority of current diagnostic markers are likely to be late stage, irreversible measures that would not be viable as outcome markers in a clinical trial (8). BOCAA (Biomarkers and Outcomes in Cerebral Amyloid Angiopathy) is a cross-sectional prospective observational feasibility study, designed with a view to guiding future larger (and potentially longitudinal) clinical trials in CAA. The aims of the project were: (1) to establish the safety, tolerability and feasibility of a dedicated clinical research protocol for use in future therapeutic trials; and (2) to discover and validate new biomarkers for CAA.

The study was funded by the Rosetrees Trust (Project No. 523286, Award No. 167120) and a Wolfson Biomarker Grant (Project No. 523289, Award No. 162022). Ethical approval was granted in October 2015 by the NHS Health Research Authority London – Dulwich Research Ethics Committee (REC reference 15/LO/1443).

4.2 Summary of study protocol

This project included 15 participants: 10 patients with CAA and 5 age matched healthy controls. Study documents (produced by the candidate) are provided in Appendix 3; this includes the standard operating procedure, information sheets for patients and healthy volunteers, screening checklist consent forms for patients and healthy volunteers, participant information and case report forms (for patients and healthy volunteers), standard operating procedures for body fluid collection (CSF and blood), the study day itinerary, and follow up questionnaires (patient and GP).

4.2.1 Clinical assessment

Data on past medical history, current medication, social history, mobility and educational attainment was collected. Participants underwent a full neurological examination, in addition to routine baseline observations (including heart rate and blood pressure), the timed get up and go test and MoCA (see Appendix 3, VIII and IX).

4.2.2 Neuropsychology

All participants underwent formal neuropsychological testing using a “vascular” battery developed by the Department of Neuropsychology.

This battery includes the following:

- Premorbid functioning (estimated using the NART)
- Current general intellectual functioning (WAIS-III, Raven’s Advanced Progressive Matrices)
- Memory; verbal and visual recall and recognition (Warrington’s Recognition Memory Test, subtests from the AMIPB)
- Naming (Graded Naming Test, Oldfield Naming Test)
- Visuo-perceptual and spatial function (subtests from the Visual Object and Space Perception battery)
- Executive and attention functions (Stroop Colour Word Test, Phonemic and Semantic Fluency, Hayling and Brixton Test, subtest from Test of Everyday Attention)
- Speed of Information Processing (Symbol Digit Modality Test, WAIS-III Symbol Search subtest)
- Mood (DASS-21)

4.2.3 Body fluid markers

CSF and blood was collected from all participants; further details on collection can be found in Appendix 3 (X and XI).

4.2.4 Imaging

Each participant was scanned using the UCLH 3-Tesla Siemens Biograph PET/MR system, using an imaging protocol based upon one currently in use for the MRC National Survey for Health and Development 1946 Birth Cohort Neuroimaging Sub-study (336). This includes:

- PET imaging using the amyloid ligand ^{18}F-florbetapir ("Amyvid")
- Neurite orientation dispersion and density imaging (NODDI)
- Pseudo-Continuous Arterial Spin Labelling
- Resting state and visual functional MRI
- Standard structural MRI sequences (including volumetric 3D T1, FLAIR and SWI)

4.2.5 Outcome data

Information on outcomes is collected from patients (but not healthy volunteers), using follow up questionnaires sent initially at 6 months and 1 year following recruitment, and then annually (for 5 years). These questionnaires (Appendix 3, XII and XIII) are sent to both patients and their GPs, and ask about functional status and clinical events including stroke, as well as other serious vascular events.

4.2.6 Patient inclusion and exclusion criteria:

Inclusion criteria:

- Adult ≥ 55 years of age, ≤100 years of age; *preferred age target 55 – 70 years*
- MMSE score ≥ 23
- mRS ≤ 3
- Fulfilling at least “probable” Modified Boston Criteria for CAA
- Competent to give informed consent

Exclusion criteria:

- Contraindications to PET or MRI scanning, or lumbar puncture

4.2.7 Control inclusion and exclusion criteria:

Inclusion criteria:

- Adult ≥ 55 years of age, ≤100 years of age; *preferred age target 55 – 70 years*
- MMSE score ≥ 23
- mRS ≤ 3
- Competent to give informed consent

Exclusion criteria:

- Contraindications to PET or MRI scanning, or lumbar puncture
- History of significant neurological disease

4.3 The BOCAA recruitment experience

4.3.1 Introduction

One of the main aims of the BOCAA study was to establish the feasibility of the research protocol, in particular with regard to patient recruitment. The inclusion and exclusion criteria were designed to reflect a patient population that could be included in a future trial; ideally this would be a population with "early" disease, although formal criteria defining this do not currently exist. One essential requirement was that patients could provide informed consent for themselves, as the lumbar puncture was regarded as a relatively invasive procedure. In a therapeutic study, the capacity to provide informed consent would be important not only at study entry but also after this, given that such a study would almost certainly require repeated visits. We also wished to select patients who would be able to participate in all aspects of the study (i.e. without contraindications to MRI or PET scanning, or lumbar puncture) and be able to physically tolerate scanning and other procedures (i.e. not limited by the sequelae of previous ICH). This was the rationale behind the cognitive (MMSE) and functional (mRS) thresholds set within the inclusion criteria.

Beyond the formal inclusion and exclusion criteria, there were other issues that influenced patient selection. This was a small pilot study, and so we wished to reduce heterogeneity within our patient group as much as possible. In order to achieve this, we selected patients who met "probable" modified Boston criteria and without evidence of other neurological diseases or pathologies; we thus excluded patients with co-existing pathologies (e.g. those with Alzheimer's disease, and those with "mixed" CAA and DPA). Additionally we did not include patients with intracranial neurosurgical implants or other devices that caused artefacts on MRI, or patients with significant visual impairment (on the basis that their visual task fMRI might be difficult to interpret). These factors might also have relevance for larger clinical studies – treatment effects might be more apparent within a less heterogenous group.

Given the large number of inclusion and exclusion criteria, an important question for BOCAA was whether it was feasible to recruit the necessary number of patients. In this section, we describe our patient recruitment methods and experience.

4.3.2 Identification methods

Two methods were used to identify potentially eligible patients. The first involved screening a prospectively collected database of CAA patients (originally collected by two other Clinical Research Associates, Andreas Charidimou and Duncan Wilson, and then by the candidate; patients listed from July 2008 onwards). This database was part of a Service Evaluation Agreement approved by the local Research Ethics Committee (joint UCL Institute of Neurology / National Hospital for Neurology and Neurosurgery); this agreement provides permission to review anonymised data from patients presenting to our stroke service with intracranial haemorrhage. The vast majority of patients in this database had attended Professor Werring's specialist intracerebral haemorrhage service, provided by the National Hospital of Neurology and Neurosurgery (NHNN), University College London Hospitals (UCLH) NHS Trust. Approximately 10% of the patients attending this service are referred from other specialist centres in England and Wales. To give an indication of attendance numbers, between January 1st 2015 and June 1st 2018, there were a total of 1192 clinic visits made by 663 individual patients. The database also includes patients referred to Professor Werring privately (details were provided by Professor Werring; these clinic lists were not routinely screened), and patients referred to him from other centres with an interest in participating in research studies.

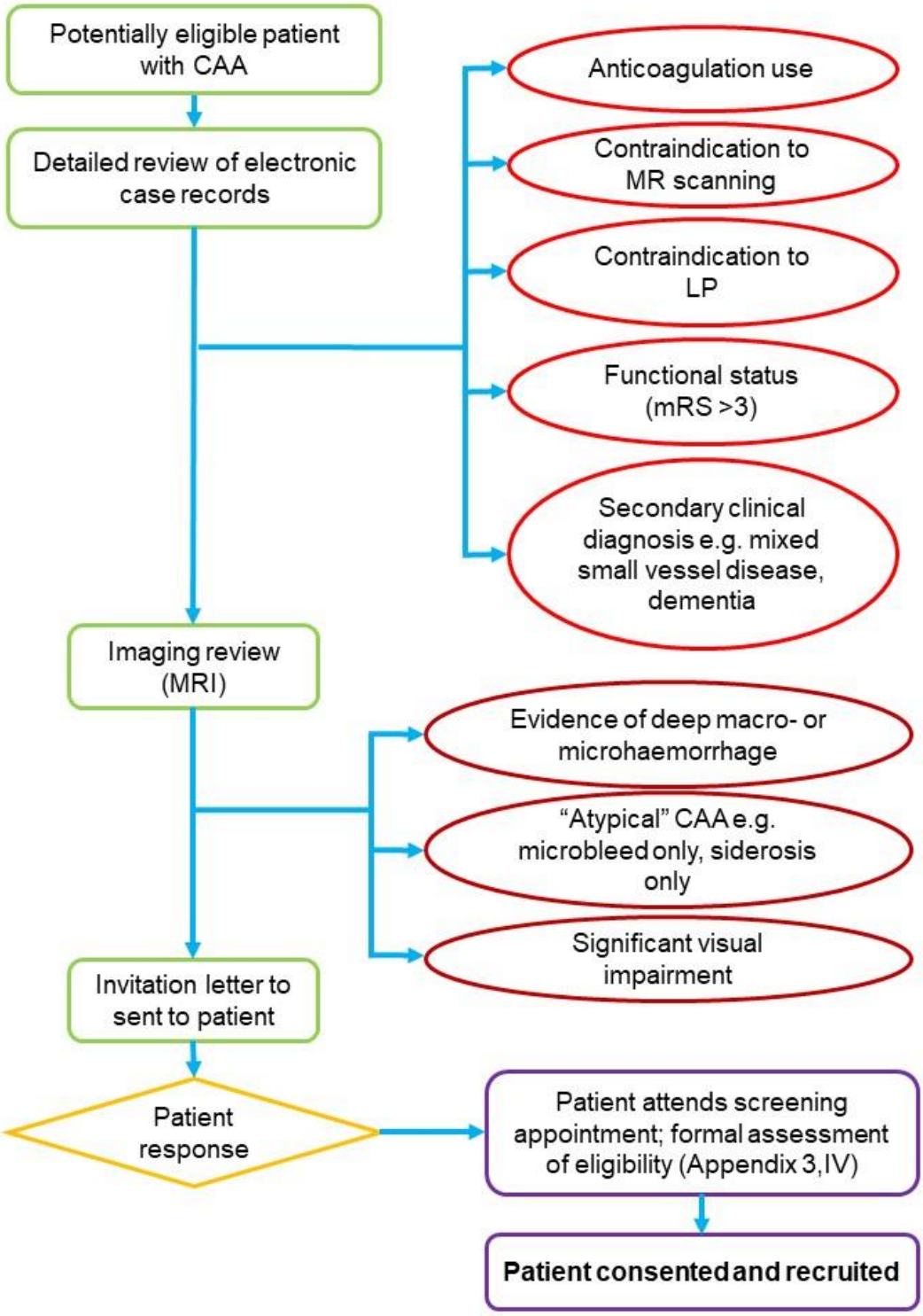
The second method was screening patients presenting with lobar ICH to the Hyperacute Stroke Unit (HASU) and Acute Brain Injury Unit (ABIU). This screening was carried out by Stroke Research Practitioners, as the study had been adopted into the North London

Stroke Clinical Research Network Portfolio. Unfortunately, this method did not successfully identify any potential participants, and 6 months after recruitment opened this method was abandoned. There were three reasons for this. Firstly, for patients admitted to and then discharged from the HASU, very few patients had MRI scans during their admission and so it was not possible to diagnose CAA. Secondly, many of the patients admitted to the ABIU were acutely unwell, often requiring intensive care support; it was not clear whether these patients would survive their brain haemorrhage, let alone recover to an extent that would allow them to meet the inclusion and exclusion criteria. Finally, nearly all patients who were discharged from the HASU (and many from the ABIU) were eventually reviewed by the specialist intracerebral haemorrhage service; if a diagnosis of CAA was made at this stage, they were then included within the CAA database.

The steps after identification of a potentially eligible patient are shown in Figure 4.3.1. Briefly, the patient's electronic case records were reviewed (by the candidate) for clinical details that would render them ineligible, after which the patient's brain MRI was reviewed (the candidate would make an initial imaging screen, before then reviewing the imaging in detail with Professor Werring). The patient was required to meet the modified Boston criteria for "probable" CAA (57); patients with features consistent with CAA but not fully meeting these criteria were excluded (e.g. those with lobar CMBs only, or cSS only), as were those with evidence of deep macro- or microhaemorrhage. If a patient appeared eligible at this stage, they were sent a letter inviting them to participate in the study, together with the patient information sheet (Appendix 3, II). This letter included a response sheet and a stamped addressed envelope. If the patient returned the response sheet stating they were willing to participate, they were invited to attend a screening appointment, where their eligibility was formally assessed (Appendix 3, IV) by the candidate. This was also an opportunity for the participant to ask questions about the study, if they had any. If the patient met the inclusion and exclusion criteria, and remained

willing to participate, they were asked to sign a consent form (Appendix 3, V) and formally recruited into the study.

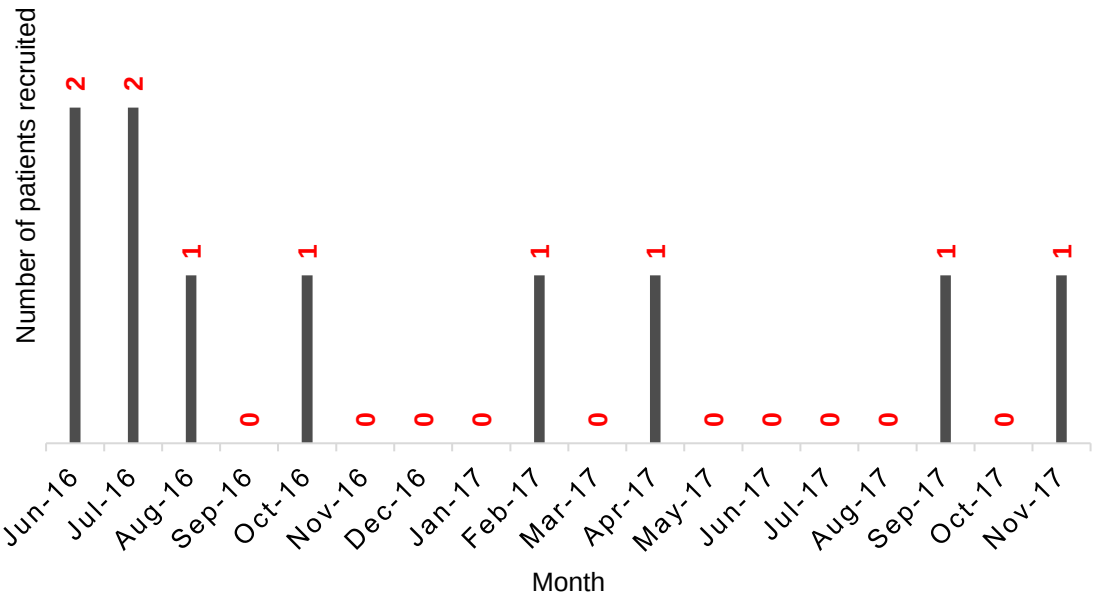
Figure 4.3.1: Flowchart detailing the process between identification of a potentially eligible patient and their recruitment



4.3.3 BOCAA recruitment – detailed breakdown

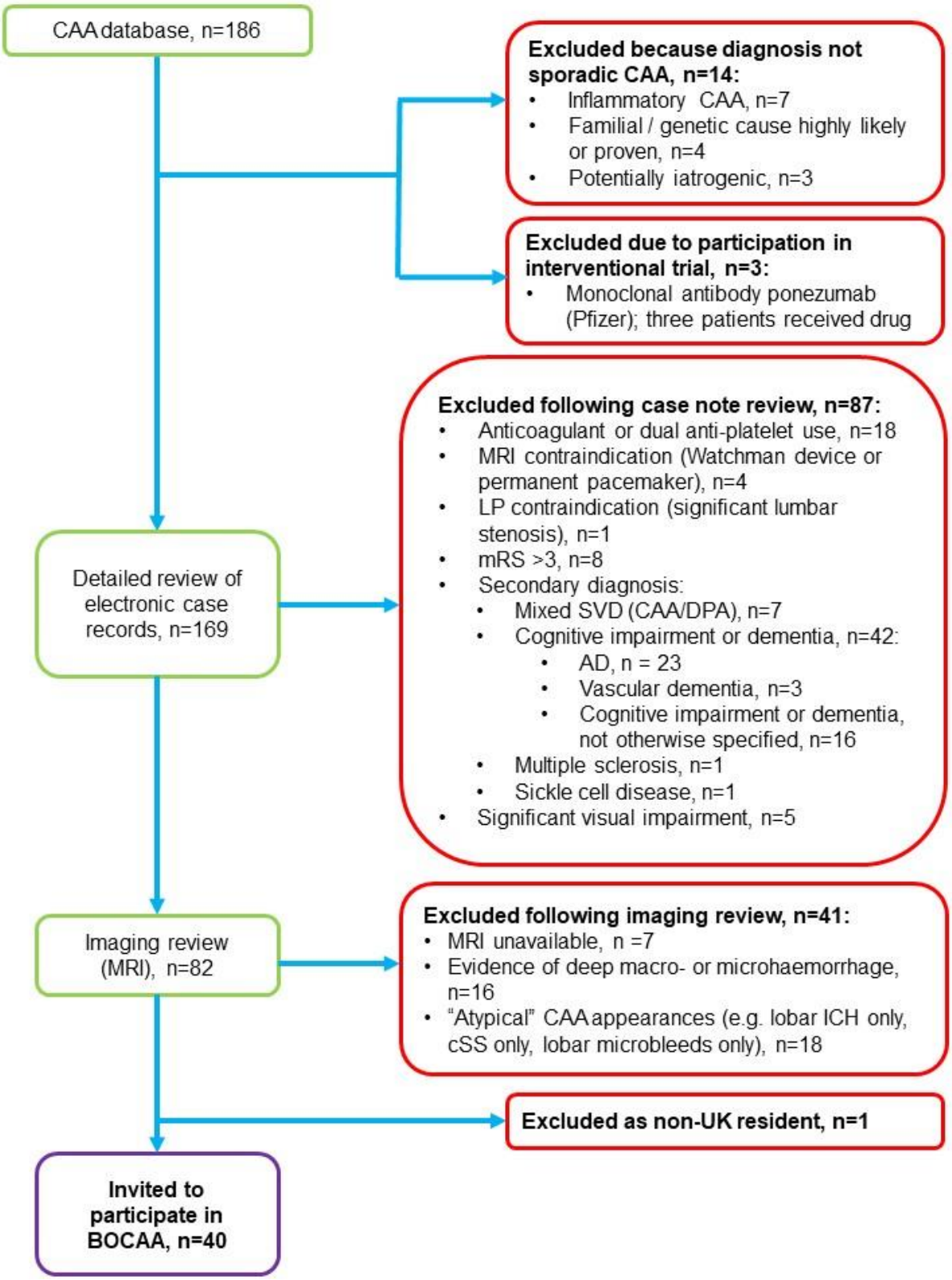
Ethical approval was given in October 2015, but recruitment did not start until late May 2016 due to delays in obtaining local R&D approval, outstanding applications to the Leonard Wolfson Experimental Neurology Centre and Leonard Wolfson Biomarker Laboratory for use of facilities, and finalisation of the PET-MR, CSF and blood processing protocols. The last patient was recruited in November 2017. Figure 4.3.2 shows the number of patients recruited per month over this time period.

Figure 4.3.2: BOCAA patient recruitment over time



The reasons for patient exclusions are shown in Figure 4.3.3. Most patients were excluded following electronic case note review, with the commonest reason being documented evidence of cognitive impairment or dementia. Of the 40 patients invited to participate, 20 patients responded to the invitation letter; of these, 10 patients declined to participate, and 10 were successfully recruited.

Figure 4.3.3: Reasons for exclusions



4.3.4 Discussion

Our experiences of patient recruitment for this small pilot study highlight some key points. Firstly, we managed to fully recruit for this study (10 CAA patients over a period of 18 months), demonstrating that this level of recruitment is feasible for a single specialist centre. Secondly, we found that recruiting patients when they presented acutely to stroke services was difficult, and that recruitment using a prospective research database was more effective. Finally, we show that a large proportion (n=146, 78.5%) of patients listed in the CAA database were ineligible for BOCAA, with the most common reason for exclusion being co-existent cognitive impairment or dementia. This suggests that the presence of cognitive impairment and dementia are relatively common in this patient cohort, a factor that we underestimated. Given that data on recruiting patients with CAA for therapeutic clinical trials is limited, this work makes an important contribution by highlighting a number of practical aspects which may inform future larger trials.

Our difficulties with recruitment after acute presentation with an ICH indicate the limitations of current biomarkers for CAA, and in particular, the need for MRI. The recent CT based “Edinburgh” criteria (58) might improve our ability to identify patients with CAA in the acute setting. Our experience also highlights the potential difficulties of recruiting from a HASU as the centre of a “hub and spoke” model (337). Although we did not formally quantify this, it is likely that a number of patients presenting to the HASU were discharged before having an MRI; these patients are likely to be those with better pre-morbid functioning as well as less severe strokes (and thus more likely to meet our inclusion criteria), and almost certainly would have an MRI when followed up by their local stroke unit (by which stage they would have been lost to our research database). The introduction of a centralised research database, compiled from standardised local databases, would allow for more efficient and co-ordinated recruitment to future CAA studies.

Whilst we did successfully fully recruit to the BOCAA study, the recruitment process took longer than originally projected; we had estimated that recruitment would take between 6 and 12 months, given that we were a tertiary centre with a specialist interest in ICH. The reason that recruitment took longer than anticipated was underestimation of the proportion of ineligible patients within the CAA database. As discussed above, the most common reason for exclusion was co-existent cognitive impairment or dementia (n=42), but patients were also excluded for not meeting the imaging criteria (n=41), and because of anticoagulant or dual antiplatelet use (n=18). This highlights the fact that the CAA patients are often older and more likely to have comorbidities, some of which may limit trial participation. It also emphasises the impact of cognitive criteria on recruitment – future studies may need to relax these criteria (i.e. be more permissive of mild cognitive impairment and/or a co-existent diagnosis of Alzheimer's disease), and as a consequence provide strategies that allow for continuing trial participation if a patient loses capacity to give informed consent.

We also found that of the 40 patients invited to participate, only 10 (25%) were eventually recruited to the study. The commonest reason for lack of participation was a failure to respond (n=20, 50% of those invited). Although we did not ask patients who declined to participate to provide their reasons for this, when a patient did provide a reason (of their own accord) it was universally that they would prefer not to have a lumbar puncture (n=3). This might reflect prevailing views that a lumbar puncture is an uncomfortable and invasive procedure, and study protocols which do not require CSF might have higher response and participation rates.

A final point for consideration is whether our recruitment strategy inadvertently introduced bias into the study. Whilst it was the pre-specified aim of BOCAA to recruit “early” CAA, our recruitment strategy will have inevitably led to the selection of patients with milder disease. Although there are advantages of this, primarily that these patients are more likely to fully participate in a future trial (especially one with a longitudinal element), only selecting patients with mild disease, especially in such a small study, could limit the identification of new biomarkers due to type II errors (false-negative results). There is also an argument that our participating patients are not representative of the “real-world” CAA, which is a heterogenous condition. These points will need to be considered when interpreting the results of the BOCAA study.

4.4 BOCAA participant baseline characteristics

The baseline demographic, clinical and imaging characteristics of the 10 patients and 5 healthy volunteers recruited to the BOCAA study are shown in Tables 4.4.1, 4.4.2 and 4.4.3. There were no significant differences in baseline clinical or demographic characteristics between the two groups (Table 4.4.1). When comparing clinical findings between patients and healthy volunteers at study entry (Table 4.4.2), there was a difference in timed get up and go test performance (mean 9.0 vs 6.8 seconds, p=0.0504), but no other measures. Details of the haemorrhagic features for the patient group are shown in Table 4.4.4.

Table 4.4.1: Baseline clinical and demographic characteristics of the BOCAA study participants

Groups were compared using t-tests (age, years of education), Mann Whitney U tests (units of alcohol per week, mRS), or Fisher's exact test (remainder).

		CAA patients	Healthy Volunteers	p value
n		10	5	-
Age, years, mean (SD)		68.6 (3.0)	64.4 (6.5)	0.1049
Sex, female, n (%)		2 (20.0)	3 (60.0)	0.251
Handedness, right, n (%)		9 (90.0)	4 (80.0)	1.000
Past Medical History				
Hypertension, n (%)		8 (80.0)	1 (20.0)	0.089
Hypercholesterolaemia, n (%)		5 (50.0)	1 (20.0)	0.580
Diabetes mellitus, n (%)		1 (10.0)	0 (0.0)	1.000
Seizures, n (%)		1 (10.0)	0 (0.0)	1.000
Migraine with aura, n (%)		6 (60.0)	4 (80.0)	0.600
Medication history				
Regular medication use, n (%)		8 (80.0)	2 (40.0)	0.251
Antihypertensive use, n (%)	0 agents	2 (20.0)	3 (60.0)	0.251
	1 agent	4 (40.0)	2 (40.0)	
	2 agents	4 (40.0)	0 (0.0)	
Statin use, n (%)		3 (30.0)	1 (20.0)	1.000
Social history				
Ex-smoker, n (%)		5 (50.0)	1 (20.0)	0.580
Units of alcohol per week, median (IQR)		7.5 (6 to 10)	14 (3 to 14)	0.8533
Years of education, mean (SD)		19.4 (3.3)	19 (3.3)	0.8276
mRS, median (IQR)		0 (0 to 0)	0 (0 to 0)	0.3006

Table 4.4.2: Clinical findings at study entry

Groups were compared using Mann Whitney U tests (MMSE and MoCA scores), or t-tests (remainder).

	CAA patients	Healthy Volunteers	p value
Height, cm, mean (SD)	174.1 (11.2)	167.8 (8.9)	0.2992
Weight, kg, mean (SD)	75.7 (14.5)	69.5 (9.9)	0.4072
BMI, kg/m^2, mean (SD)	24.9 (3.3)	24.6 (2.7)	0.9019
Systolic blood pressure, mmHg, mean (SD)	134.1 (10.6)	125.8 (10.6)	0.1771
Diastolic blood pressure, mmHg, mean (SD)	77.7 (4.8)	77.6 (6.6)	0.9735
Heart rate, beats per minute, mean (SD)	69.5 (11.9)	62.8 (7.6)	0.2758
Timed get up and go test, seconds, mean (SD)	9.0 (2.0)	6.8 (1.5)	0.0504
MMSE score, median (IQR)	29 (28 to 30)	30 (29 to 30)	0.1688
MoCA score, median (IQR)	26.5 (25 to 29)	28 (27 to 29)	0.2909

Table 4.4.3: Imaging findings at study entry

Rating for all structural markers were completed using methods detailed in Section 2.3.

		CAA patients	Healthy Volunteers
WMH (Fazekas score), median (IQR)	dWMH	1 (1 to 2)	0 (0 to 0)
	pvWMH	3 (2 to 3)	1 (1 to 1)
Lacunes, n (%)		0 (0.0)	1 (20.0)
MTA grade, median (IQR)		1 (1 to 1)	0 (0 to 0)
GCA grade, median (IQR)		1 (0 to 1)	2 (1 to 2)
cSS, n (%)	None	3 (30.0)	5 (100.0)
	Focal	3 (30.0)	0 (0.0)
	Disseminated	4 (40.0)	0 (0.0)
Lobar CMBs, median (IQR)		3.5 (2 to 25)	0 (0.0)

Table 4.4.4: Haemorrhagic details for the BOCAA patient group

ID	Age	Sex	History of TFNE	History of previous symptomatic ICH	Presence of cSS	Presence of lobar microbleeds
BOC01	70	F	-	-	Disseminated	Yes
BOC02	64	M	-	Yes (multiple)	-	Yes
BOC04	67	M	-	Yes (single)	Focal	Yes
BOC05	72	M	Yes	-	Disseminated	Yes
BOC08	69	F	Yes	-	Disseminated	Yes
BOC10	67	M	Yes	-	Disseminated	Yes
BOC14	67	M	-	Yes (multiple)	-	Yes
BOC15	66	M	-	Yes (multiple)	-	Yes
BOC16	74	M	Yes	-	Focal	Yes
BOC17	70	M	-	-	Focal	Yes

4.5 Body fluid biomarkers for CAA

4.5.1 Introduction

The limitations of current biomarkers for CAA have been discussed in detail in the Introduction (Section 1); briefly, the diagnosis of CAA is possible using the clinico-radiological Boston criteria (57, 59), but nearly all of the imaging features associated with CAA are likely to be irreversible markers of late stage disease (8). This is likely to limit their use as outcome markers for therapeutic clinical trials, where early, more dynamic and potentially reversible measures are needed (8). CAA shares a pathological moiety (the Aβ protein) with AD, and there is both clinical and neuropathological overlap between the two conditions (14); a biomarker, or biomarkers, which could better differentiate between these two Aβ pathologies would be important from both clinical and research perspectives.

Body fluid biomarkers, either from the cerebrospinal fluid (CSF) or blood, might avoid the limitations of imaging markers. A review of our current understanding of fluid markers in CAA is described in Section 1.5.2; to summarise, most of this data has focussed on Aβ and tau measures in CAA. Results suggest that patients with CAA have lower Aβ-40 (than patients with AD and healthy controls) and Aβ-42 (than healthy controls), with total tau and phospho-tau levels that are intermediate between healthy controls and patients with AD (122, 150-152, 338-340). Data from memory clinic populations (often including patients with AD) suggests that patients with imaging features suggestive of CAA (lobar CMBs, cSS) may show a similar “CAA” CSF profile for Aβ and tau (154, 155, 157, 158, 341). However, there is limited data on the smaller amyloid proteins in CAA (for example, Aβ-38, sAPPα and sAPPβ).

A number of newer body fluid biomarkers have recently been described, which might be of interest in CAA. Neurofilaments are a key component of the neuronal axonal

cytoskeleton (342), and both CSF and blood levels of the smallest of these, neurofilament light (NFL) (343), are increased in a number of neurological conditions, including various dementias (344-346), multiple sclerosis (347, 348), stroke (349-352) and cerebral small vessel disease (353-355), amongst others. Variants in the microglial gene *TREM2* (Triggering Receptor Expressed on Myeloid Cells 2) are associated with an increased risk of developing AD (356, 357), and levels of the soluble form of the TREM2 protein (sTREM2) are elevated in the CSF of patients with AD (358-362), as well as in patients with multiple sclerosis (363, 364). Neurogranin is a neuronal post-synaptic protein expressed in dendritic spines (365, 366) and elevated CSF concentrations are observed in AD (365-377) and acute ischaemic stroke (378). However, whilst NFL, sTREM2 and neurogranin are promising new biomarkers for AD, it is not clear whether they are markers of the presence of any Aβ pathology (parenchymal or vascular), or if they are specific for parenchymal Aβ and AD alone. Finally, elevations in CSF ferritin have been observed in AD (379, 380); the haemorrhagic associations of CAA, together with evidence that the CSF of patients with CAA may have elevated red blood cell counts (381), make this another promising marker for investigation. As yet, none of these markers have yet been evaluated in CAA; the identification of different profiles for these two Aβ conditions could provide insight into the mechanisms resulting in vascular versus parenchymal deposition.

The aim of this project was to identify new body fluid biomarkers for CAA, primarily using CSF. Our objective was to establish which measures were different between three participant groups: patients with CAA, age matched healthy volunteers and patients with AD.

4.5.2 Methods

4.5.2.1 Patient selection

We included participants from the BOCAA study; the selection criteria and recruitment methods are described in detail in Sections 4.2 and 4.3. The BOCAA study included 10 patients with CAA, and 5 age matched healthy volunteers.

We included further samples collected by the Specialist Cognitive Disorders Service (based at NHNN, UCLH NHS Trust). We included 20 samples from patients with AD, and another 5 samples from age matched healthy volunteers. Patients with AD presented with "typical" (382) amnestic symptoms, were aged ≥ 55 years, and had a final diagnosis (on the basis of clinical assessment, imaging and CSF) that was in keeping with Alzheimer's disease; additionally, all imaging was reviewed for the presence of CMBs and cSS, and patients with these features were not included (in order to avoid patients with mixed CAA and AD pathology). Samples for age matched healthy volunteers were included if their final diagnosis, made on the basis of clinical assessment, imaging and CSF, was not one of dementia or any other neurodegenerative condition. Additional inclusion criteria included age ≥ 55 years, MMSE score ≥ 23, mRS ≤ 3, and the absence of a prior history of significant neurological disease (i.e. consistent with the inclusion and exclusion criteria for healthy volunteers in BOCAA). MR imaging was reviewed for evidence of previous infarction (including lacunes), CMBs, and cSS; samples were only included in the absence of these features. Atrophy (MTA, GCA) and WMH were also assessed on brain imaging, and those with evidence of moderate or severe grades of MTA, GCA or WMH were also excluded.

4.5.2.2 Body fluid analysis

CSF and blood samples were processed and analysed by staff at the Leonard Wolfson Biomarker Laboratory; the methods for this section were provided by Martha Foiani and Jamie Toombs (both affiliated with the UK Dementia Research Institute at UCL**,** and the Department of Molecular Neuroscience, UCL Institute of Neurology).

CSF was collected, processed and stored at -80°C according to standardised procedures (383). Briefly, samples were transported to lab and centrifuged within 30 minutes from collection. CSF was centrifuged at 1750g for 5 minutes at 4^{o}C, and blood at 1800g for 5 minutes at room temperature; samples were then aliquoted and stored at -80^{o}C until testing.

Amyloid measures

Aβ-38, Aβ-40 and Aβ-42 were measured by electrochemiluminescence (ECL) using a Meso Scale Discovery V-PLEX Aβ peptide panel 1(6E10) kit, according to the manufacturer's instructions. Briefly, samples were diluted 1:2 with diluent 35 and added in duplicate to microplate wells coated with mouse monoclonal peptide specific capture antibodies for human Aβx-38/x-40/x-42. Samples were incubated with anti-Aβ (amino acids 1-16 epitope) antibody (6E10 clone) as the detection antibody conjugated with an electrically excitable SULFO-TAG. Concentrations were calculated from ECL signal using a four-parameter logistic curve fitting method with the MSD Workbench software package. Intra-assay CVs (co-efficient of variance) were less than 10%. All samples were measured on the same day by a single operator using the same reagents.

sAPPα and sAPPβ were measured by ECL using a Meso Scale Discovery sAPPα/sAPPβ Kit, according to manufacturer instructions. Briefly, samples were diluted 1:4 with 1% Blocker A and added in duplicate to microplate wells coated with mouse

(sAPPα) and rabbit (sAPPβ) monoclonal peptide specific capture antibodies. Samples were incubated with anti-sAPPα and anti-sAPPβ detection antibodies conjugated with an electrically excitable SULFO-TAG. Concentrations were calculated from ECL signal using a four-parameter logistic curve fitting method with the MSD Workbench software package. Intra-assay CVs were less than 20%. All samples were measured on the same day by a single operator using the same reagents.

Tau markers (total tau and phospho-tau)

The levels of CSF total tau and phospho-tau$_{(181P)}$ were determined using a sandwich ELISA (INNOtest® hTAU-Ag P-Tau$_{(181P)}$; Fujirebio Europe N.V., Ghent, Belgium) constructed to measure both normal tau and phosphorylated tau. Briefly, for the hTAU Ag assay, tau protein is captured from CSF samples by a monoclonal anti-tau antibody (AT120) bound to a microtiter plate. Captured tau is detected with two biotinylated tau-specific monoclonal antibodies (HT7 and BT2). Similarly, for the total tau assay, phospho-tau$_{(181P)}$ is captured from CSF samples by anti-tau antibody HT7 bound onto a microtiter plate. Captured phospho-tau$_{(181P)}$ is detected with a biotinylated monoclonal anti-phospho-tau antibody (AT270). In both assays, peroxidase-labelled streptavidin and tetramethylbenzidine (TMB) substrate are also added. Peroxidase catalyzed hydrolysis produces a colorimetric signal. Sample concentrations are extrapolated from a standard curve, fitted using a 4-parameter logistic algorithm. Samples with intra-assay CVs more than 20% were excluded.

Neurofilament light (NFL)

CSF NFL was measured by UMAN diagnostics ELISA, according to manufacturer instructions. Briefly, samples were diluted 1:2 with sample diluent and added in duplicate to microplate wells coated with a monoclonal capture antibody specific for NFL. Samples were incubated with a biotinylated NFL-specific monoclonal detection antibody. The detection complex was completed with the addition of horseradish peroxidase-labelled

streptavidin and tetramethylbenzidine (TMB) substrate. Peroxidase catalyzed hydrolysis produces a colorimetric signal. Sample concentrations were extrapolated from a standard curve, fitted using a 4-parameter logistic algorithm. Intra-assay CVs were less than 10%. Samples were run on two different days by different operators.

The methods for determining serum NFL concentrations have been described previously (384); the Simoa HD-1 analyser platform (Quanterix) was used to measure NfL concentrations using the manufacturer's NfL reagent kit and in accordance with manufacturer instructions. Briefly, serum samples were diluted fourfold and then incubated with paramagnetic beads coated with anti-NfL antibodies and biotinylated detector antibodies. Beads were then washed and combined with a conjugate of streptavidin-β-galactosidase. This enzyme binds to the biotinylated antibodies, labelling the captured protein molecules of interest. Following an additional wash, beads were suspended in a resorufin-β-D-galactopyranoside (RGP) substrate and transferred into an array of sealed microwells. If the enzyme-labelled protein of interest is bound to a bead it hydrolyses RGP and produces sufficient fluorescent signal to be detected by the analyser, even if only a single molecule is bound. The analyser measures the proportion of 'positive' wells containing beads bound to at least one molecule of interest (giving a 'digital' output proportional to the amount of the protein of interest in the sample when it is at low concentrations) and also the total fluorescent signal from all wells (giving an 'analogue' output proportional to the amount of the protein of interest present in the sample when it is at higher concentrations). Finally, concentrations were measured with a four-parameter logistic curve fit. All samples had a CV less than 10% and were analysed with one batch of reagents.

sTREM2 measurements

CSF samples were analysed using an immunoassay protocol adapted from a previously published protocol (385). Streptavidin-coated 96-well plates (Meso-Scale Discovery

(MSD), Rockville, MA, USA) were blocked overnight at 4°C in block buffer (0.5% bovine serum albumin (BSA) and 0.05% Tween 20 in PBS; pH 7.4). The plates were then incubated with the biotinylated polyclonal goat anti-human TREM2 capture antibody (0.25 µg/ml; BAF1828, R&D Systems, Minneapolis, MN, USA) diluted in block buffer, shaking for 1 hour at room temperature. They were subsequently washed five times with wash buffer (0.05% Tween 20 in PBS) and incubated for 2 hours shaking at room temperature with 50µL per well of either the standard curve constructed from recombinant human TREM2 protein (11084-H08H-50, Sino Biological Inc., Beijing, China) diluted in assay buffer (0.25% BSA and 0.05% Tween 20 in PBS; pH 7.4) to produce concentrations ranging between 4000pg/ml and 62.5pg/ml, or CSF samples diluted 1 in 4 in assay buffer. Standards and CSF samples were assayed in duplicate. Plates were again washed five times with wash buffer before incubation for 1 hour shaking at room temperature with the detection antibody, monoclonal mouse anti-human TREM2 antibody (1µg/ml; (B-3): sc373828, Santa Cruz Biotechnology, Texas, USA), diluted in block buffer. After five additional washing steps, plates were incubated with the secondary antibody (SULFO-TAG-labelled goat anti-mouse secondary antibody, R32AC-5, MSD) and incubated shaking for 1 hour in the dark. Lastly, plates were washed three times with wash buffer then twice in PBS alone. The electrochemical signal was developed by adding MSD Read buffer T 4x (R92TC-2, MSD) diluted 1 in 2, and the light emission measured using the MSD Sector Imager 6000. The concentration of sTREM2 was calculated using a five-parameter logistic curve fitting method with the MSD Workbench software package. Intra-assay CVs were less than 10%, and all samples were measured on the same day by a single operator using the same reagents.

Neurogranin

Neurogranin was measured with the EUROIMMUN Elisa (EQ6551-9601-L) according to manufacturer's instructions. Briefly, samples were incubated with biotinylated monoclonal anti-Neurogranin antibody, followed by addition to microplate wells coated

with monoclonal antibodies specific for human neurogranin truncated at P75. Finally, streptavidin peroxidase conjugate was added to initiate the colour-changing reaction. The concentration of neurogranin was calculated using a five-parameter logistic curve fitting method with the MSD Workbench software package. Intra-assay CVs were less than 10%, and all samples were measured on the same day by a single operator using the same reagents.

Ferritin

Ferritin was measured a latex fixation test according to manufacturer's instructions; these methods have been described previously (386). All samples were measured on the same day by a single operator using the same reagents.

4.5.2.3 Statistics

Statistical analysis was performed by the candidate using Stata (Version 15.1). Median and interquartile range values were calculated for each biomarker, and, given the non-normal distribution of the data, comparisons between groups were made using the Kruskal-Wallis test. If a significant difference was identified (defined as $p<0.05$), Dunn's test was used for post-hoc comparisons, and a Bonferroni correction (resultant p value multiplied by 3) was applied.

In order to perform age adjusted analyses, we used quantile regression (comparing group medians), and calculated predicted medians. We then performed post-hoc pairwise comparisons of the predicted medians, with a Bonferroni correction. Statistical significance was defined as $p<0.05$.

4.5.3 Results

We included 20 patients with AD, 10 patients with CAA and 10 healthy volunteers (HV) in this analysis; baseline characteristics are shown in Table 4.5.1. Patients with CAA were older (mean age 68.6 years, compared with 62.5 years in the AD group and 62.2 years in the HV group), and those in the AD group had a lower MMSE (median score 24, compared with 29 for the CAA and HV groups).

4.5.3.1 Aβ-38

There was a significant difference between the three groups (Figure 4.5.1; p=0.0019). In post-hoc comparisons, patients with CAA had significantly lower CSF Aβ-38 than both AD (corrected p=0.0015) and HV (corrected p=0.0042) groups. There was no difference between AD and HV groups (corrected p=1.00).

In the age adjusted quantile regression (Table 4.5.2) there was a significant difference between the three groups (p=0.0005); pairwise comparisons of the predicted medians found significant differences between AD and CAA (Bonferroni corrected 95% CI, 590.0 to 2360.0 pg/ml), and the HV and CAA (Bonferroni corrected 95% CI, 651.3 to 2646.6 pg/ml) groups.

4.5.3.2 Aβ-40

There was a significant difference between the three groups (Figure 4.5.2; p=0.0001).Patients with CAA had significantly lower CSF Aβ-40 than both AD (corrected p=0.00003) and HV (corrected p=0.0006) groups. There was no difference between AD and HV groups (corrected p=1.00).

The age adjusted quantile regression (Table 4.5.2) identified a significant difference between the three groups (p=0.0002). Pairwise comparisons of the predicted medians

found significant differences between AD and CAA (Bonferroni corrected 95% CI, 1509.7 to 5562.1 pg/ml) and the HV and CAA (Bonferroni corrected 95% CI, 1780.1 to 6348.2 pg/ml) groups.

4.5.3.3 Aβ-42

There was a significant difference between the three groups (Figure 4.5.3; p=0.0001). In post-hoc comparisons, patients with CAA had significantly lower CSF Aβ-42 than both AD (corrected p=0.0006) and HV (corrected p=0.00003) groups. Patients with AD had lower CSF Aβ-42 than the HV group, but this was not statistically significant after Bonferroni correction (corrected p=0.1368).

In age adjusted quantile regression (Table 4.5.2), there was a significant difference between the three groups (p=0.0005). Pairwise comparison of the predicted medians found significant differences between CAA and HV groups (Bonferroni corrected 95% CI, 167.5 to 620.2 pg/ml) and the HV and AD groups (Bonferroni corrected 95% CI, HV vs AD, 60.6 to 404.0 pg/ml). The difference between the CAA and AD groups did not reach statistical significance (Bonferroni corrected 95% CI, -39.2 to 362.3 pg/ml).

4.5.3.4 sAPPα

There was a significant difference between the three groups (Figure 4.5.4; p=0.0082). In post-hoc analyses, patients with CAA had significantly lower CSF sAPPα than both AD (corrected p=0.0066) and HV (corrected p=0.0126) groups. There was no difference between AD and HV groups (corrected p=1.00).

In age adjusted quantile regression, there was no significant difference between the three groups (Table 4.5.2).

4.5.3.5 sAPPβ

There was a significant difference between the three groups (Figure 4.5.5; p=0.0092). In post-hoc pairwise comparisons, patients with CAA had significantly lower CSF sAPPβ than both AD (corrected p=0.0060) and HV (corrected p=0.0183) groups. There was no difference between AD and HV groups (corrected p=1.00).

In age adjusted quantile regression (Table 4.5.2) there was a significant difference between the CAA group and the AD and HV groups (p=0.0236). Pairwise comparison of the predicted medians found significant differences between CAA and AD (Bonferroni corrected 95% CI, 2.8 to 94.3 pg/ml), and the CAA and HV groups (Bonferroni corrected 95% CI, 7.3 to 110.3 pg/ml).

4.5.3.6 Total tau

There was a significant difference between the three groups (Figure 4.5.6; p=0.0001). In post-hoc analyses, patients with CAA had significantly lower CSF total tau than AD patients (corrected p=0.0042). There was no statistically significant difference between the CAA and HV groups (corrected p=0.3534) groups. Patients with AD had significantly higher CSF total tau than the HV group (corrected p=0.00003).

In the age adjusted quantile regression (Table 4.5.2), there was a significant difference between the three groups (p=0.0002). Pairwise comparisons of the predicted medians found significant differences between the AD and CAA (Bonferroni corrected 95% CI, 82.5 to 632.2 pg/ml) and the HV and AD (Bonferroni corrected 95% CI, -645.5 to -175.4 pg/ml) groups. There was no difference between the HV and CAA groups (Bonferroni corrected 95% CI, -362.9 to 256.7 pg/ml).

4.5.3.7 Phospho-tau

There was a significant difference between the three groups (Figure 4.5.7; p=0.0001). In post-hoc analyses, patients with CAA had significantly lower CSF phospho-tau than AD patients (corrected p=0.0141). There was no statistically significant difference between the CAA and HV groups (corrected p=0.2616) groups. Patients with AD had significantly higher CSF phospho-tau than the HV group (corrected p=0.00003).

In the age adjusted quantile regression (Table 4.5.2), there was a significant difference between the groups (p=0.0003). Pairwise comparisons of predicted medians found significant differences between the AD and CAA (Bonferroni corrected 95% CI, 10.7 to 71.2 pg/ml) and the HV and AD groups (Bonferroni corrected 95% CI, -69.0 to -17.2 pg/ml). Again, there was no significant difference between the HV and CAA groups (Bonferroni corrected 95% CI, -36.3 to 31.9 pg/ml).

4.5.3.8 Neurofilament light (NFL)

CSF

There was a significant difference between the three groups (Figure 4.5.8; p=0.0003). In post-hoc analyses, patients with CAA had significantly higher CSF NFL than the HV group (corrected p=0.00003). There was no statistically significant difference between the CAA and AD groups (corrected p=0.1497) groups. Patients with AD had significantly higher CSF NFL than the HV group, but this did not reach statistical significance (corrected p=0.0051).

In age adjusted quantile regression, there was no significant difference between the three groups (Table 4.5.2).

Serum

There was a significant difference between the three groups (Figure 4.5.9; p=0.0283). In post-hoc analyses, patients with CAA (mean 38.0 pg/ml) had significantly higher serum NFL than the HV group (corrected p=0.0117). There was no statistically significant difference between the CAA and AD groups (corrected p=0.2721) groups. Patients with AD had higher serum NFL than the HV group, but this did not reach statistical significance (corrected p=0.1245).

In age adjusted quantile regression, there was no significant difference between the three groups (Table 4.5.2).

4.5.3.9 CSF soluble TREM2

There was no significant difference between the three groups (Figure 4.5.10; p=0.519); similar results were seen using age adjusted quantile regression (Table 4.5.2).

4.5.3.10 Neurogranin

There was a significant difference between the three groups (Figure 4.5.11; p=0.0118). In post-hoc analyses, there was no difference between CAA and AD (corrected p=0.0759) or the HV group (corrected p=0.7188). Patients with AD had significantly higher levels of CSF neurogranin than the HV group (corrected p=0.0084).

Age adjusted quantile regression, identified a significant difference between the three groups (Table 4.5.2), but no significant differences were identified in pairwise comparisons of the adjusted medians.

4.5.3.11 Ferritin

There was a significant difference between the three groups (Figure 4.5.12; p=0.0136). In post-hoc analyses, patients with CAA had significantly higher CSF ferritin than patients with AD (corrected p=0.0060) and the HV (corrected p=0.0483) group. There was no significant difference between difference healthy volunteers and AD patients (corrected p=1.00).

In age adjusted quantile regression, there was no significant difference between the three groups (Table 4.5.2).

Table 4.5.1: Comparison of baseline characteristics and biomarkers by group

p values were obtained using one-way ANOVA (age), chi squared (sex) tests, or Kruskal-Wallis (remainder).

	CAA (n=10)	AD (n=20)	HV (n=10)	p value
Age, years, mean (SD)	68.6 (3.0)	62.5 (4.1)	62.2 (5.4)	0.0014
Sex, female, n (%)	2 (20%)	11 (55%)	5 (50%)	0.180
MMSE, median (IQR)	29 (28 to 30)	24 (19.5 to 26)	29 (29 to 30)	0.0001
Biomarkers				
Aβ-38, pg/ml, median (IQR)	1485.5 (1349.0 to 2452.5)	2739.5 (2359.0 to 3264.8)	2839.3 (2148.5 to 3274.5)	0.0019
Aβ-40, pg/ml, median (IQR)	3147.8 (2940.5 to 4136.5)	6465.0 (5761.0 to 7328.0)	6887.0 (5076.0 to 7597.0)	0.0001
Aβ-42, pg/ml, median (IQR)	115.0 (91.35 to 134.0)	322.8 (263.8 to 375.8)	520.3 (279.5 to 813.5)	0.0001
sAPPα, pg/ml, median (IQR)	88.6 (67.9 to 100.0)	115.0 (99.0 to 136.5)	117.0 (105.0 to 136.0)	0.0082
sAPPβ, pg/ml, median (IQR)	85.8 (66.3 to 104.0)	117.8 (101.7 to 142.0)	123.8 (97.5 to 143.5)	0.0092
Total tau, pg/ml, median (IQR)	316.2 (247.2 to 439.8)	656.9 (497.3 to 869.4)	249.7 (206.4 to 265.9)	0.0001
Phospho-tau, pg/ml, median (IQR)	62.1 (45.8 to 72.1)	92.8 (73.6 to 112.3)	49.5 (42.0 to 52.4)	0.0001
CSF NFL, pg/ml, median (IQR)	2783.7 (2384.5 to 8376.6)	2370.4 (1917.0 to 2727.6)	1466.3 (1148.5 to 1628.2)	0.0003
Serum NFL, pg/ml, median (IQR)	29.0 (19.5 to 55.8)	21.3 (19.6 to 28.5)	16.4 (12.6 to 21.7)	0.0283
sTREM2, pg/ml, median (IQR)	7038.4 (6242.0 to 9233.0)	6579.4 (5640.5 to 8115.9)	7961.7 (6125.4 to 9784.6)	0.5188
Neurogranin, pg/ml, median (IQR)	432.0 (348.7 to 490.8)	564.9 (454.4 to 702.1)	408.6 (308.5 to 431.0)	0.0118
Ferritin, ng/ml, median (IQR)	10.1 (8.4 to 14.0)	7.8 (6.4 to 9.2)	8.0 (6.9 to 9.0)	0.0136

Table 4.5.2: Age adjusted quantile regression (comparing medians) and predicted medians

Biomarker	Group	β (SE)	Predicted median, pg/ml (95% CI)	p value
Aβ-38, pg/ml	CAA	*Reference group*	1260.5 (679.2 to 1841.8)	0.0005
	AD	1475.0 (369.7)	2735.5 (2366.9 to 3104.1)	
	HV	1648.9 (416.7)	2909.4 (2392.3 to 3426.5)	
Aβ-40, pg/ml	CAA	*Reference group*	2994.2 (1663.4 to 4325.0)	0.0002
	AD	3535.9 (846.4)	6530.1 (5686.2 to 7373.9)	
	HV	4064.1 (954.1)	7058.3 (5874.5 to 8242.2)	
Aβ-42, pg/ml	CAA	*Reference group*	138.8 (6.9 to 270.7)	0.0005
	AD	161.6 (83.9)	300.4 (216.7 to 384.0)	
	HV	393.8 (94.5)	532.6 (415.3 to 650.0)	
sAPPα, pg/ml	CAA	*Reference group*	96.6 (71.3 to 121.9)	0.4685
	AD	18.9 (16.1)	115.5 (99.5 to 131.6)	
	HV	20.0 (18.1)	116.6 (94.1 to 139.1)	
sAPPβ, pg/ml	CAA	*Reference group*	70.6 (40.6 to 100.6)	0.0236
	AD	48.6 (19.1)	119.1 (100.1 to 138.1)	
	HV	58.8 (21.5)	129.4 (102.7 to 156.1)	
Total tau, pg/ml	CAA	*Reference group*	324.3 (143.8 to 504.9)	0.0002
	AD	357.3 (114.8)	681.7 (567.2 to 796.1)	
	HV	-53.1 (129.4)	271.3 (110.7 to 431.8)	
Phospho-tau, pg/ml	CAA	*Reference group*	56.0 (36.1 to 75.9)	0.0003
	AD	40.9 (12.6)	96.9 (84.3 to 109.5)	
	HV	-2.2 (14.2)	53.8 (36.2 to 71.5)	
CSF NFL, pg/ml	CAA	*Reference group*	2858.3 (1530.8 to 4185.9)	0.3575
	AD	-510.7 (844.3)	2347.7 (1505.9 to 3189.4)	
	HV	-1313.3 (951.7)	1545.1 (364.1 to 2726.0)	
Serum NFL, pg/ml	CAA	*Reference group*	27.9 (17.6 to 38.1)	0.4027
	AD	-2.5 (6.5)	25.4 (18.9 to 31.9)	
	HV	-8.9 (7.3)	19.0 (9.8 to 28.1)	
sTREM2, pg/ml	CAA	*Reference group*	6991.9 (4998.8 to 8985.0)	0.5453
	AD	-76.9 (1267.6)	6915.0 (5651.3 to 8178.8)	
	HV	1098.6 (1428.9)	8090.5 (6317.6 to 9863.4)	
Neurogranin, pg/ml	CAA	*Reference group*	396.1 (252.2 to 540.1)	0.0213
	AD	209.8 (91.5)	605.9 (514.6 to 697.2)	
	HV	24.0 (103.2)	420.1 (292.0 to 548.1)	
Ferritin, ng/ml	CAA	*Reference group*	9.3 (7.1 to 11.4)	0.4654
	AD	-1.7 (1.4)	7.6 (6.2 to 9.0)	
	HV	-0.9 (1.6)	8.4 (6.4 to 10.3)	

Table 4.5.3: Summary of fluid biomarker findings

Blue square indicates HV value; triangles are relative to this. Red triangles are lower than the HV value, green triangles are higher than the HV value. The presence of two triangles indicates a statistically significant difference (corrected p<0.05); one triangle indicates corrected p<0.10.

	Unadjusted comparisons (Kruskal-Wallis / post-hoc Dunn's test)			Age adjusted (quantile regression, comparison of medians)		
	CAA	AD	HV	CAA	AD	HV
Aβ-38	▼▼	■	■	▼▼	■	■
Aβ-40	▼▼	■	■	▼▼	■	■
Aβ-42	▼▼	■	■	▼▼	▼▼	■
sAPPα	▼▼	■	■	■	■	■
sAPPβ	▼▼	■	■	▼▼	■	■
Total tau	■	▲▲	■	■	▲▲	■
Phospho-tau	■	▲▲	■	■	▲▲	■
CSF NFL	▲▲	▲▲	■	■	■	■
Serum NFL	▲▲	■	■	■	■	■
sTREM2	■	■	■	■	■	■
Neurogranin	▲	▲▲	■	■	■	■
Ferritin	▲▲	■	■	■	■	■

Figure 4.5.1: CSF Aβ-38

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

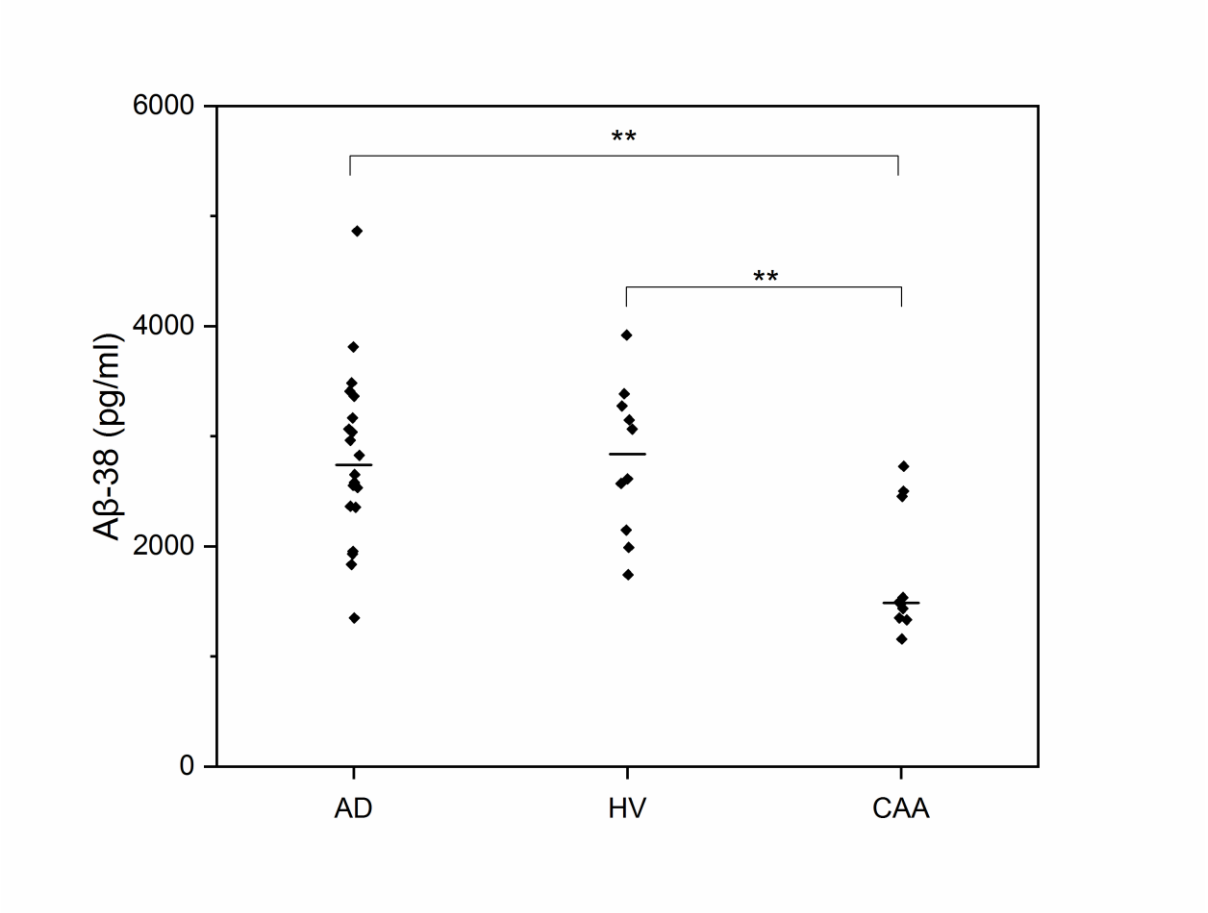


Figure 4.5.2: CSF Aβ-40

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

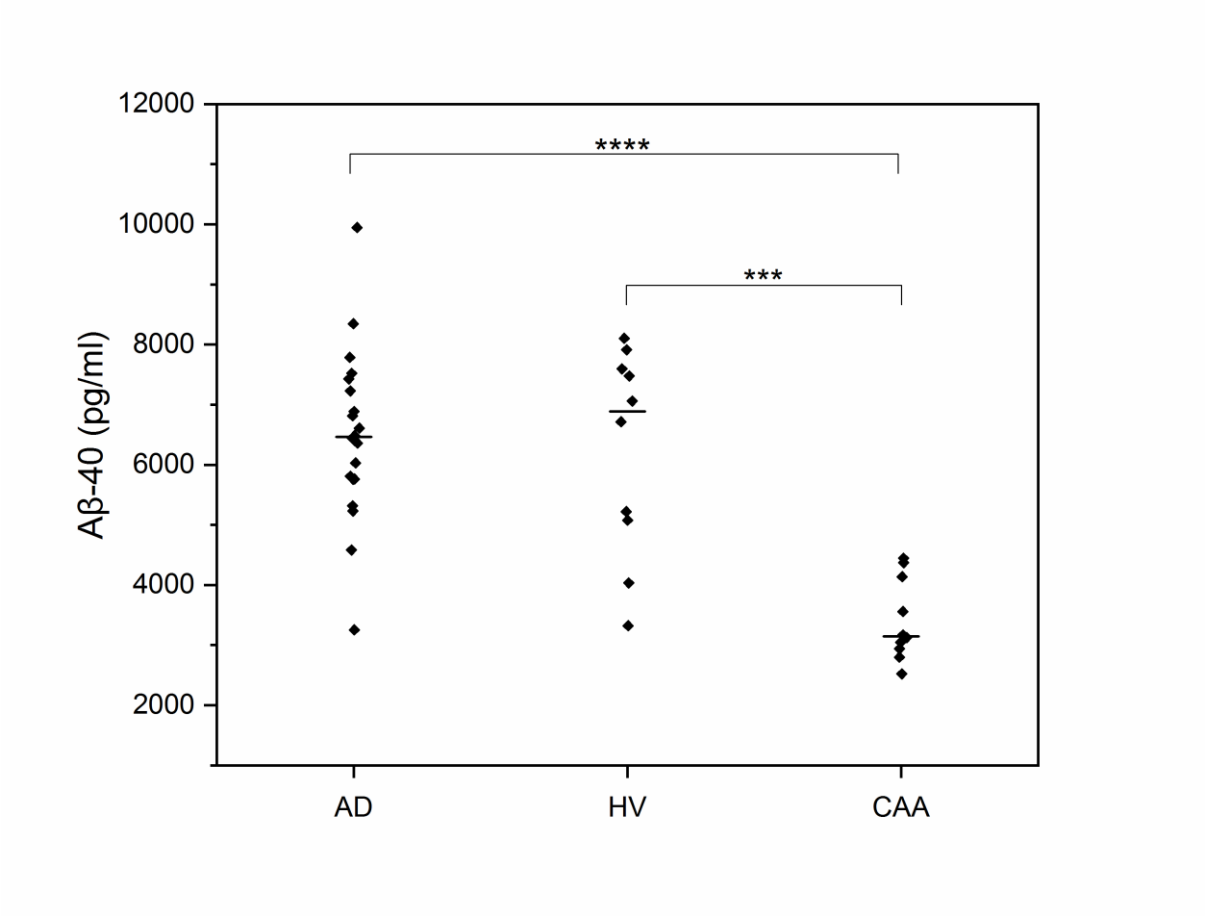


Figure 4.5.3: CSF Aβ-42

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

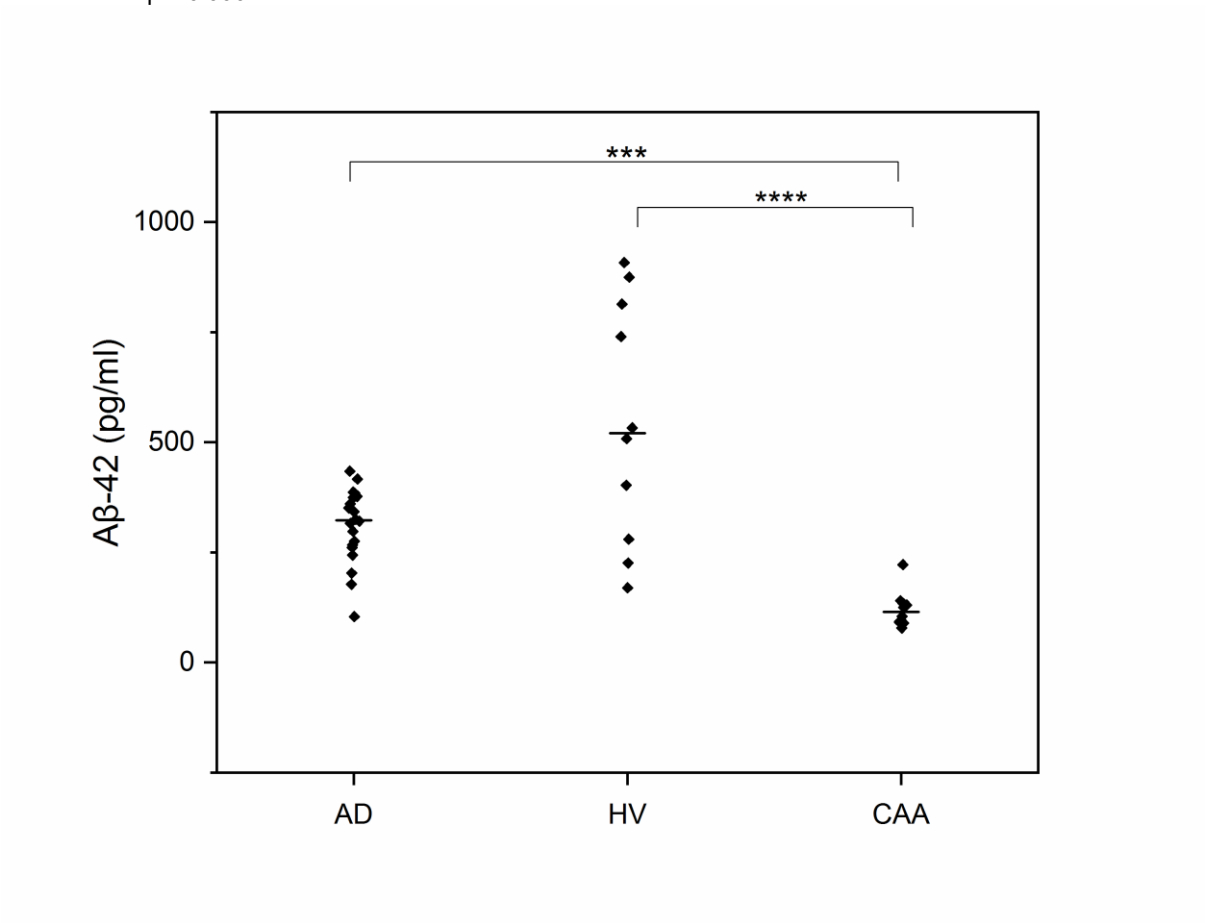


Figure 4.5.4: CSF sAPPα

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

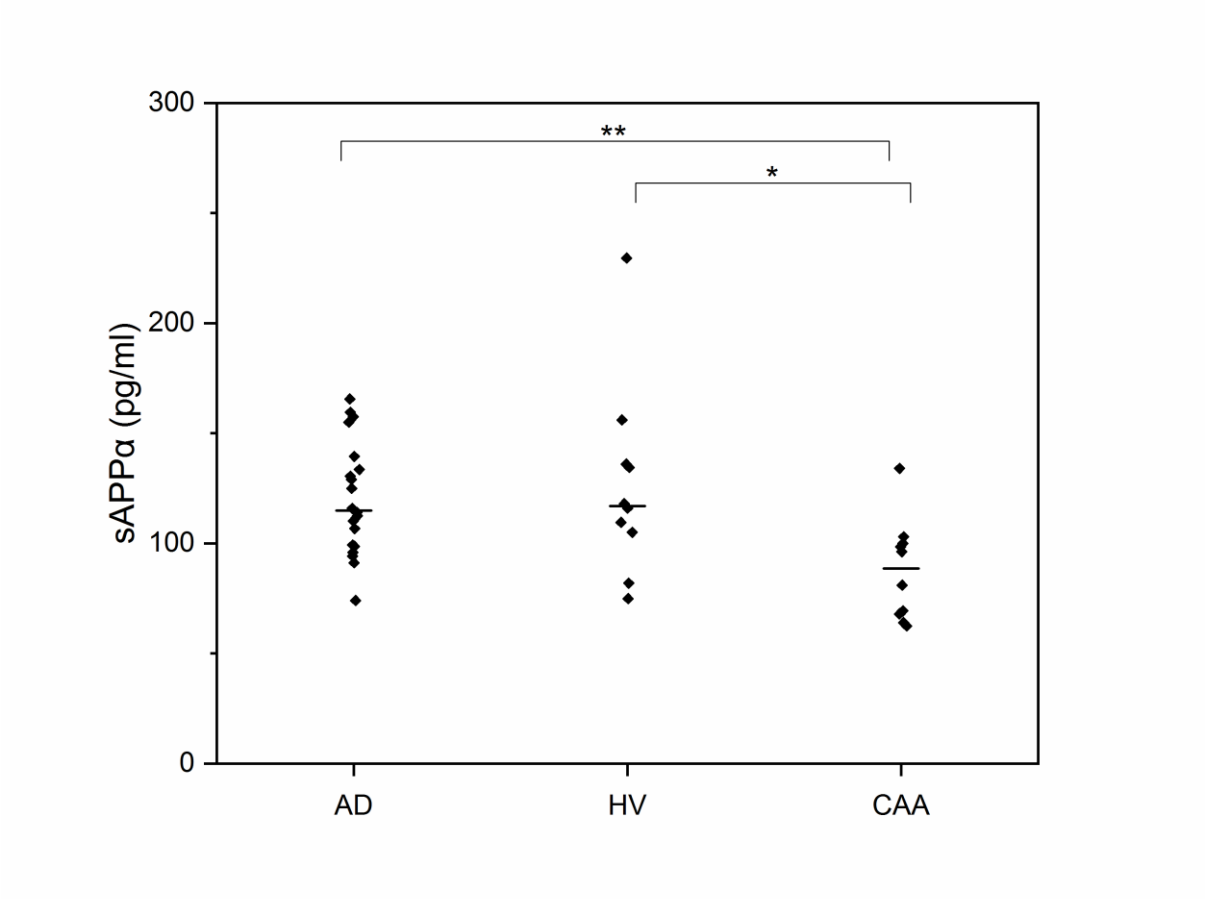


Figure 4.5.5: CSF sAPPβ

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

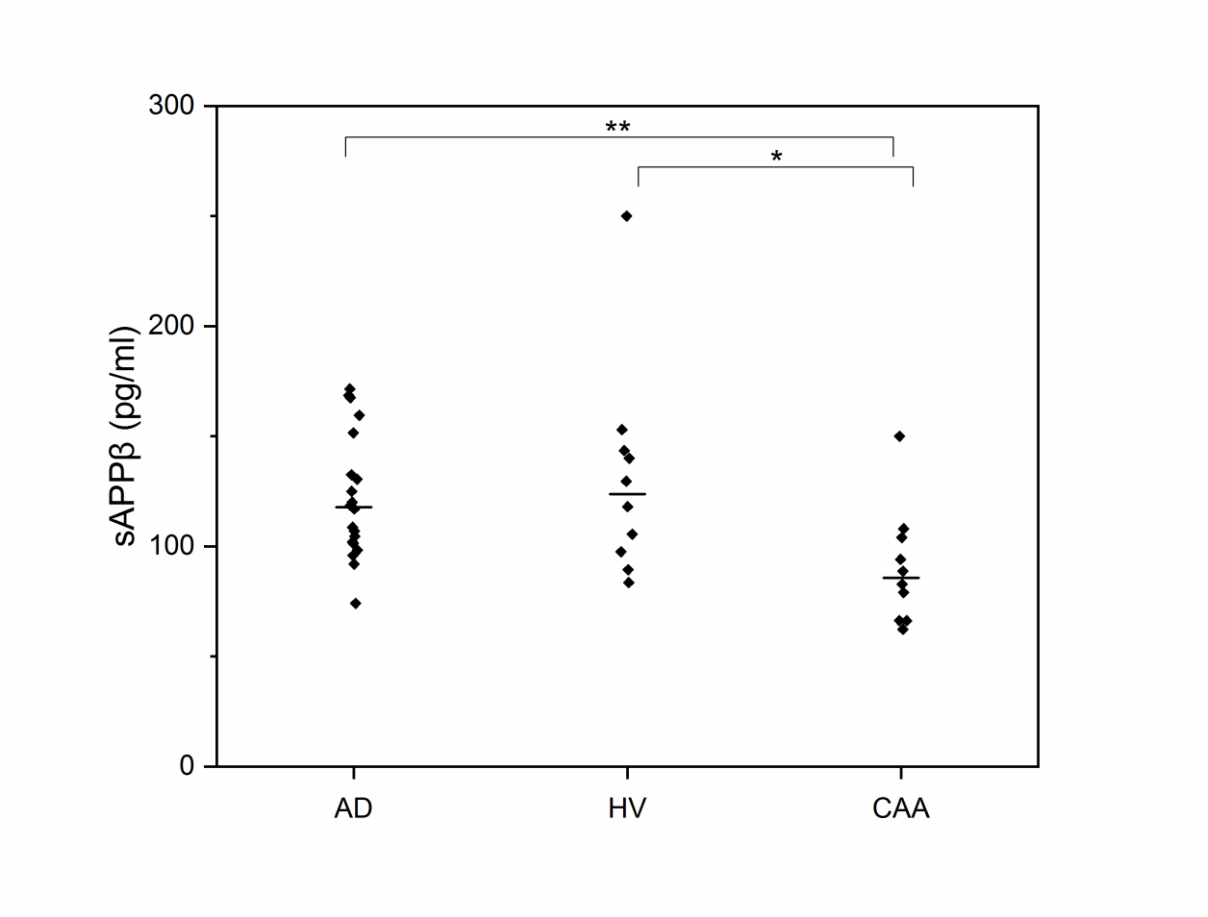


Figure 4.5.6: CSF total tau (t-Tau)

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

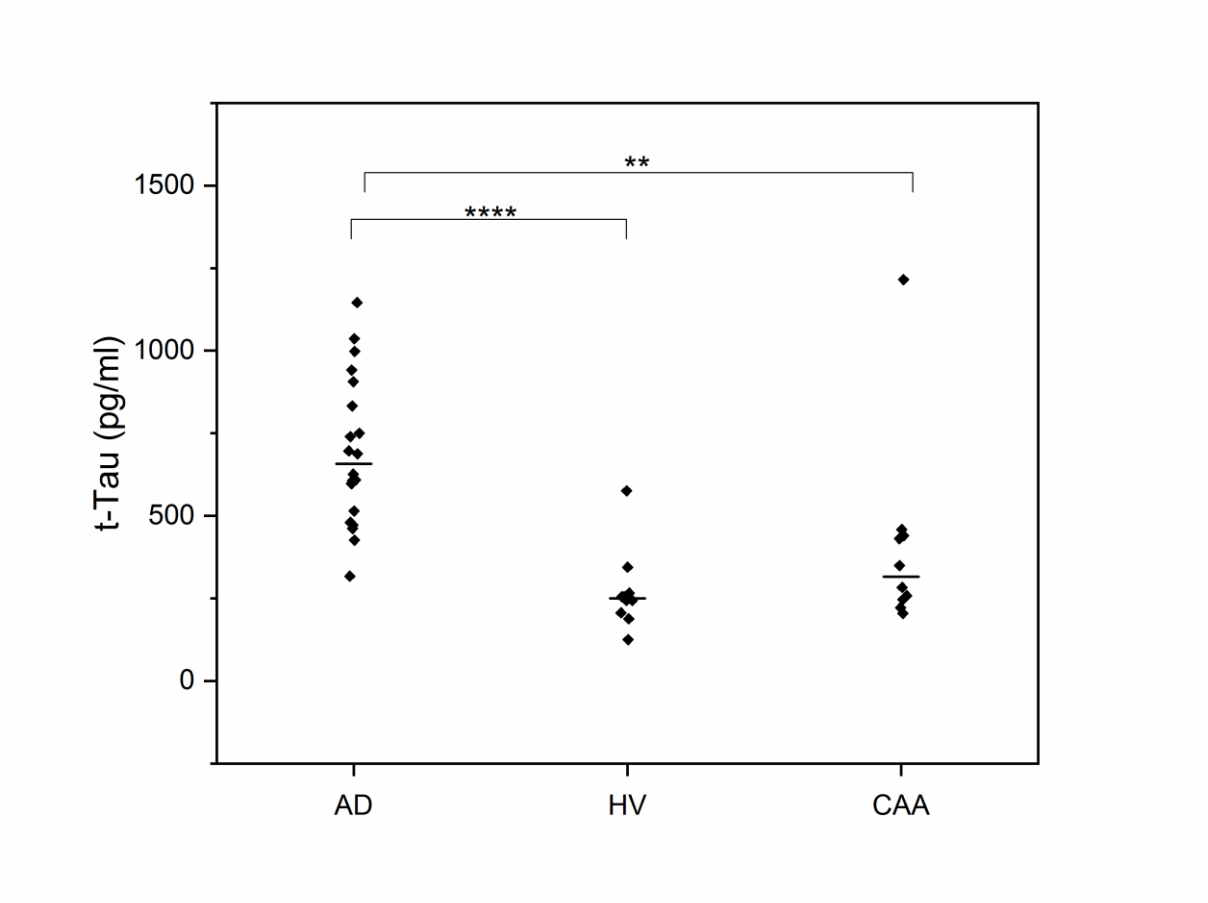


Figure 4.5.7: CSF phospho-tau (p-Tau)

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

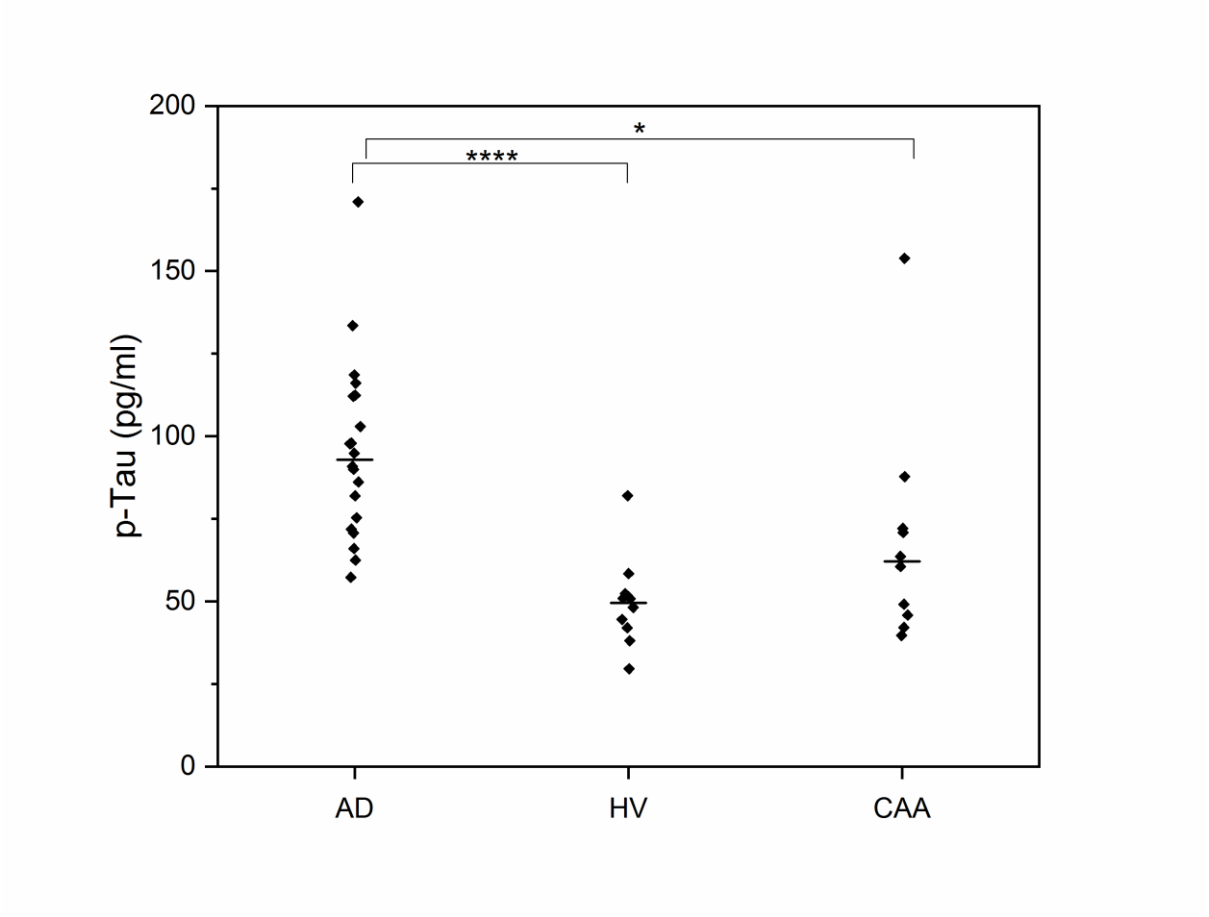


Figure 4.5.8: CSF neurofilament light (NFL)

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

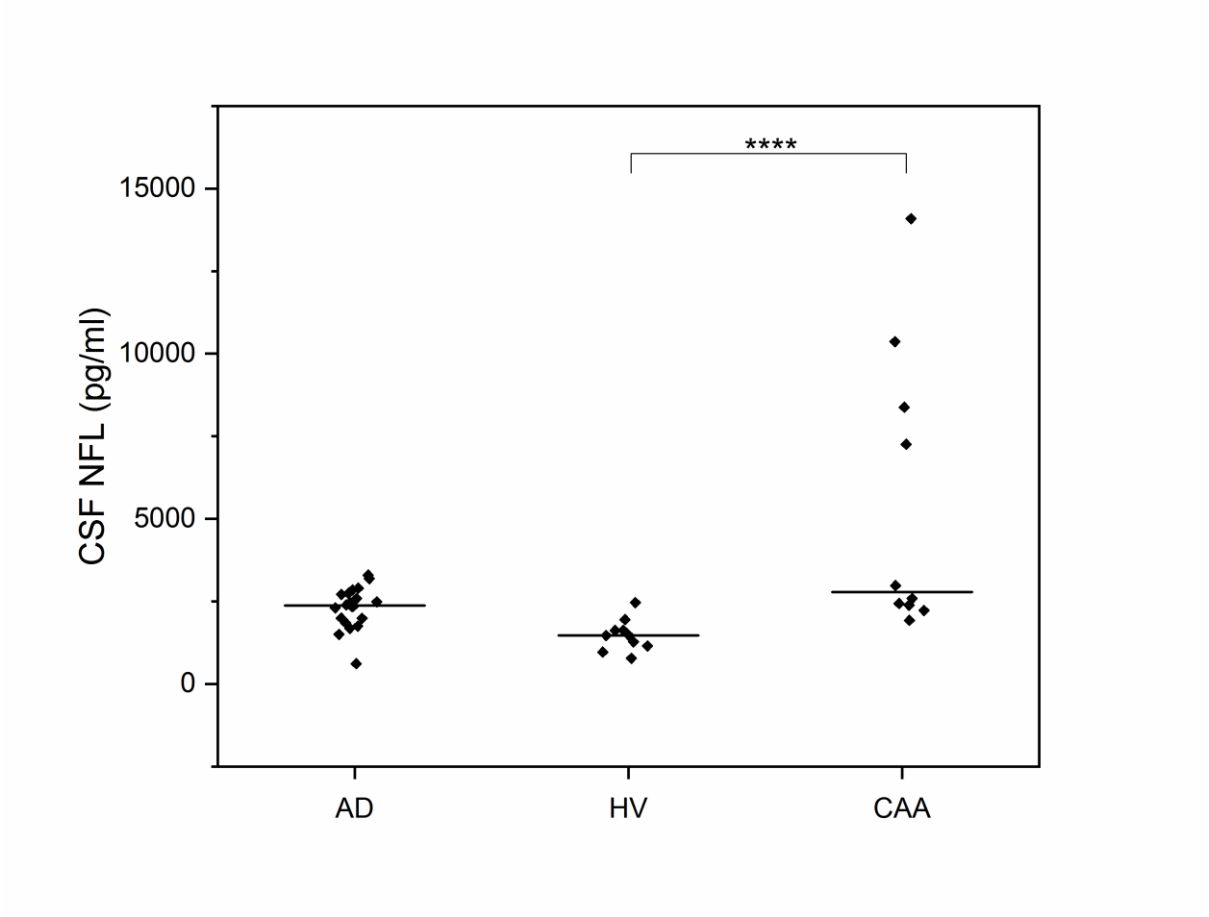


Figure 4.5.9: Serum neurofilament light (NFL)

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

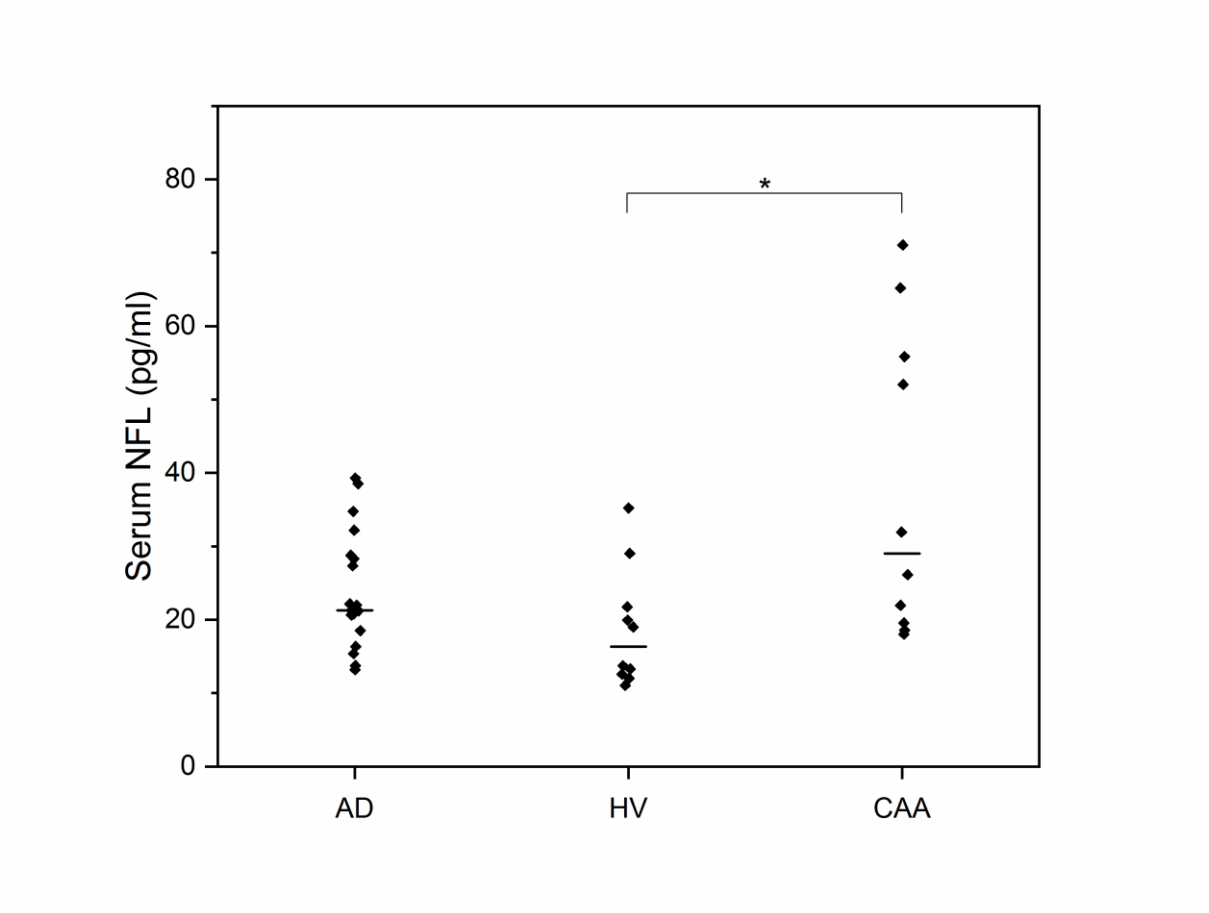


Figure 4.5.10: CSF soluble TREM2

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

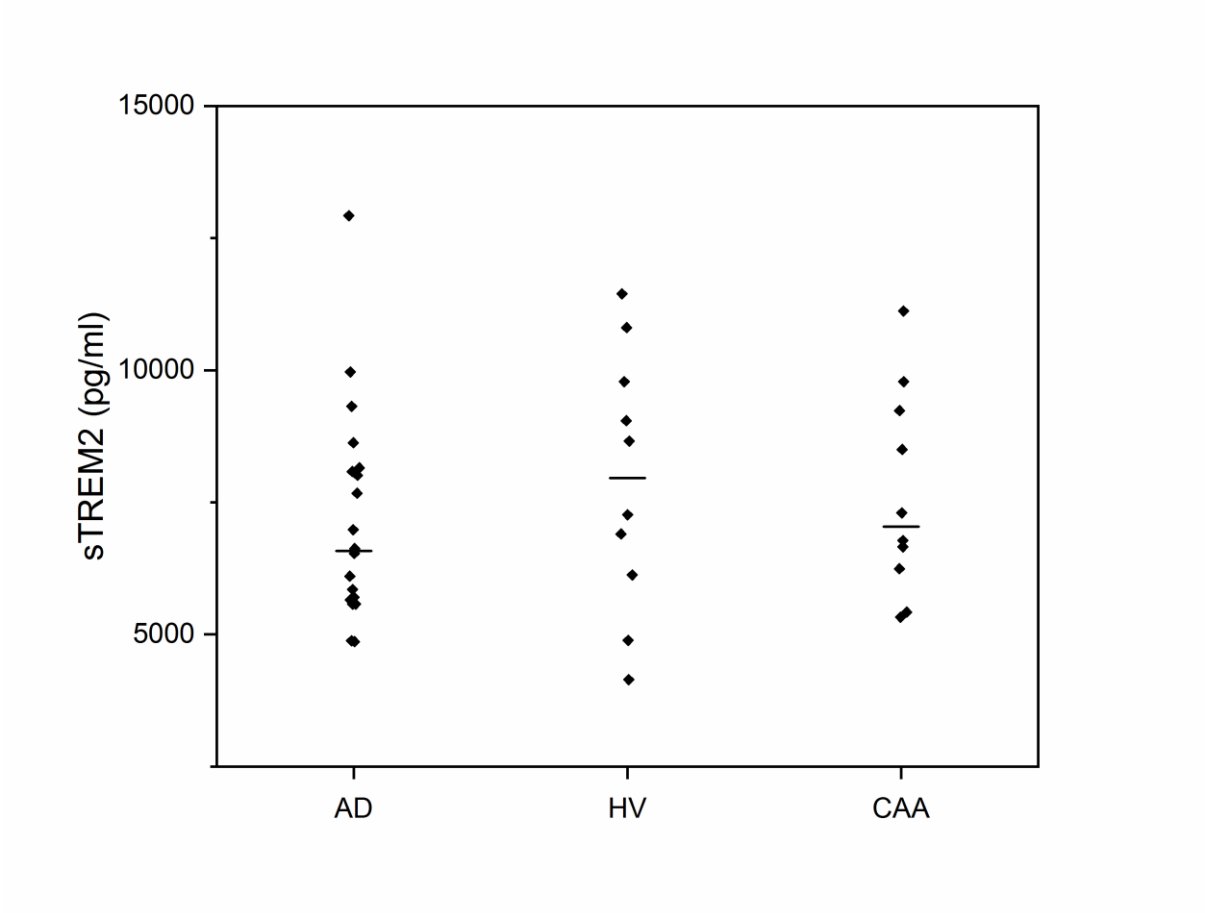


Figure 4.5.11: CSF neurogranin

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001

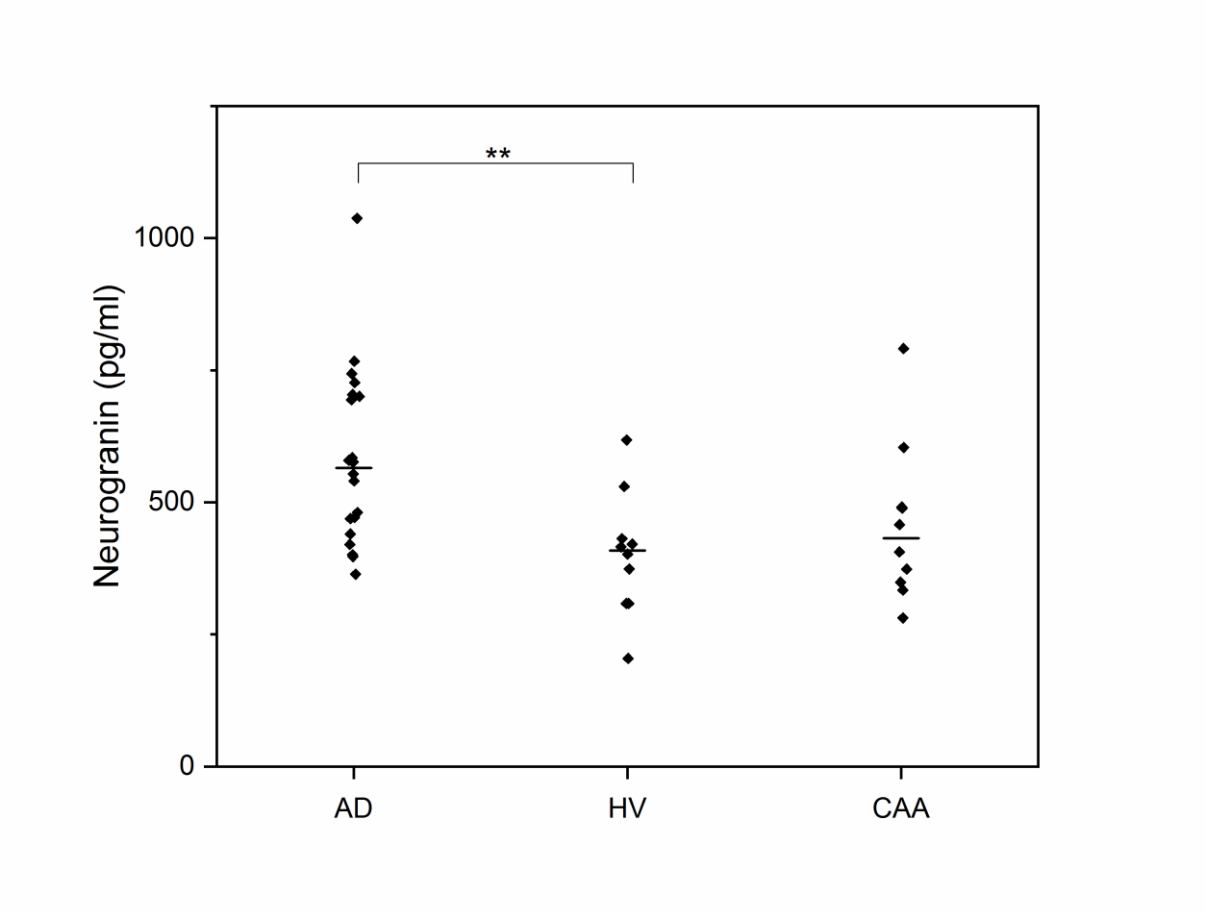
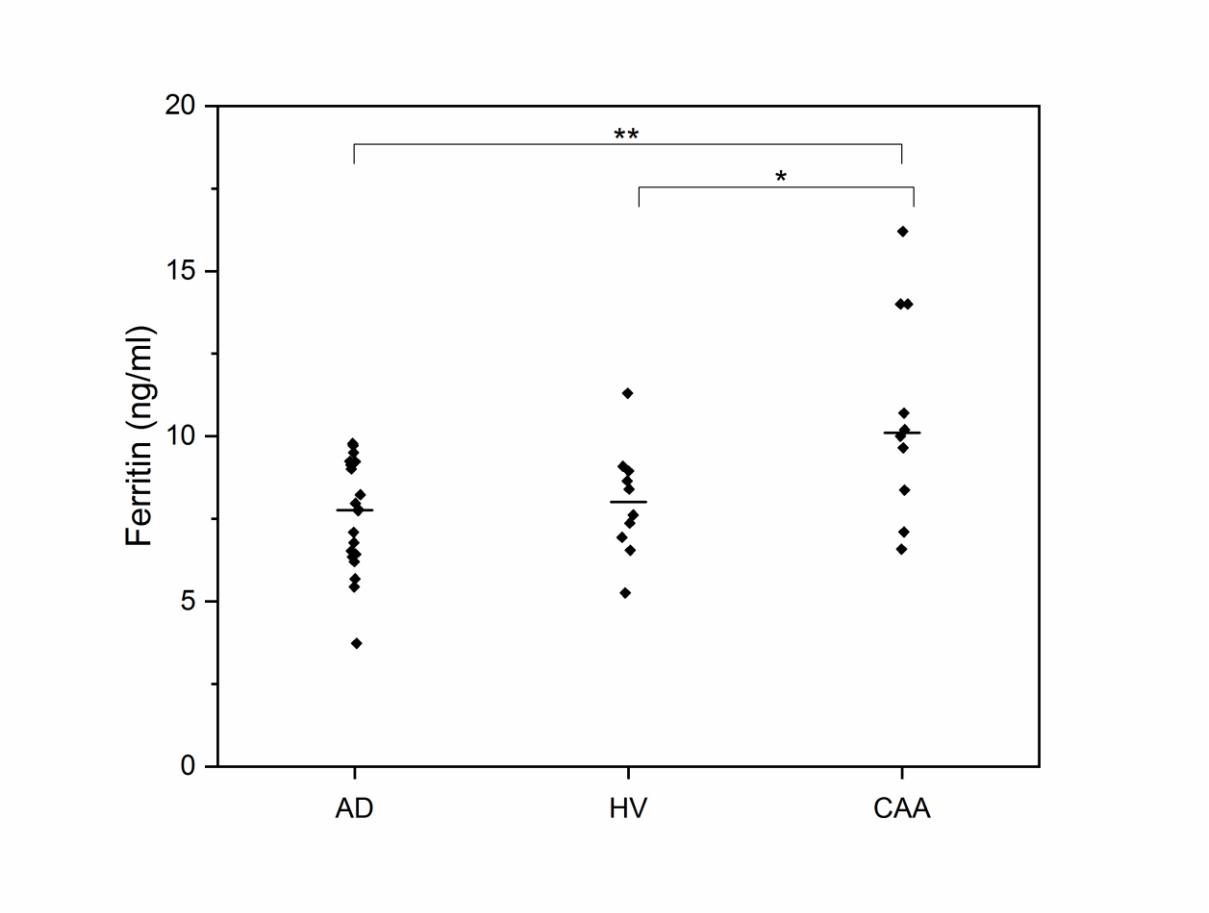


Figure 4.5.12: CSF ferritin

Horizontal line indicates median value per group. Each diamond indicates an individual data point. p values are derived from post-hoc Dunn's test and have been Bonferroni corrected.

* indicates p ≤ 0.05
** indicates p ≤ 0.01
*** indicates p ≤ 0.001
**** indicates p ≤ 0.0001



4.5.4 Discussion

Our main findings are summarised in Table 4.5.3. We found that patients with CAA had a distinctive body fluid profile compared with the HV and AD groups. In unadjusted analyses, patients with CAA showed lower levels of all amyloid components measured (Aβ-38, Aβ-40, Aβ-42, sAPPα and sAPPβ), and higher levels of serum NFL and ferritin than both other groups. Patients with AD had higher total tau and phospho-tau than both HV and CAA groups; CSF NFL was increased in both CAA and AD groups relative to the HV group. Neurogranin was higher in patients with AD than HV, and there was no difference in sTREM2 between the three groups. In age adjusted analyses, differences for the CAA group remained for Aβ-38, Aβ-40, Aβ-42, and sAPPβ; for the AD group, Aβ-42 was significantly different from HV (but not the CAA group). Our findings for amyloid and tau markers in CAA are in keeping with data reported previously by other groups (122, 150-152, 338-340); however, we extend this earlier work further by demonstrating that that Aβ-40 is not the only amyloid species to be reduced in CAA, and by providing new data on biomarkers (including NFL, sTREM2, neurogranin and ferritin) which are yet to be quantified in CAA.

Our finding that most Aβ species are reduced in patients with CAA supports the protein-elimination failure hypothesis for CAA (76), which proposes that CAA results due to failed Aβ clearance via intramural peri-arterial drainage pathways (387). The processing pathway from amyloid precursor protein (APP) to pathological Aβ is well described (Figure 4.5.13) (388), and has been studied most frequently in the context of AD and the "amyloid hypothesis" (389, 390). However, CAA differs from AD in that parenchymal Aβ plaques are predominantly composed of Aβ-42, whereas the vascular Aβ deposits in CAA are a mixture of Aβ-40 and Aβ-42, with the former being more common (150, 391). The reduced levels of CSF Aβ-40 and Aβ-42 previously described in patients with CAA have been hypothesised to be secondary to "selective trapping" of both these species in the vasculature, in contrast with AD, where only Aβ-42 is found ("trapped") in the

parenchyma (150). This hypothesis is supported by observations from patients treated with anti-Aβ immunotherapy. There is post-mortem evidence that although parenchymal Aβ plaques were reduced after treatment, the amount of vascular amyloid increased, with more vessels identified as containing both Aβ-40 and Aβ-42 (392). This change was associated with higher microbleed counts (another feature of CAA), and the authors hypothesised that anti-Aβ immunotherapy solubilises the Aβ protein, which then cannot be cleared (for unknown reasons), resulting in CAA (392). These pathological findings are supported by clinical descriptions of ARIA (amyloid-related imaging abnormalities), where patients treated with anti-Aβ immunotherapy develop imaging features associated with CAA (115, 393, 394). Our finding of reductions in Aβ-38 and sAPPβ (and sAPPα, in our unadjusted analyses) are novel, and might suggest that these elements are also trapped within the cerebral vasculature, potentially the result of a more generalised protein clearance failure. Finally, these results show the CSF Aβ profiles in AD and "haemorrhagic" CAA are different, which could mean that these two diseases occur due to failures of Aβ elimination at different stages; one could hypothesise that in AD, failure occurs at the solubilisation stage (i.e. plaque breakdown), whereas in CAA, plaque breakdown has occurred successfully but peri-vascular clearance has failed. Failure of both plaque breakdown and clearance would then result in the commonly encountered mixed AD / CAA phenotype (14). Post-translational modifications of the amyloid protein have been described (388), and these may also contribute to the differences in CSF profile observed.

Our results provide new information on non-amyloid biomarkers in CAA. We found significant elevations in total tau, phospho-tau and neurogranin in patients with AD, but did not find any differences in patients with CAA compared with healthy controls. This is in contrast with other studies which have found that total tau and phospho-tau levels in CAA are higher than controls but lower than patients with AD (150-153), and may reflect our small sample size. Pathological aggregation of tau protein is important in AD and

other neurodegenerative diseases (395, 396); tau aggregation is thought to result in synaptic dysfunction and subsequent neuronal loss, and in AD, it is tau (rather than Aβ) pathology that most closely correlates with cognition (396). Cognitive impairment is a recognised feature of CAA (87) and whilst there is evidence that CAA is associated with atrophy (presumably secondary to neuronal loss (55)), cognitive impairment in these patients might be secondary to other mechanisms, such as network disruption (102) or impaired blood flow responses (98). Our CSF findings suggest that synaptic dysfunction is a less prominent feature of CAA compared with AD, and that these markers might be useful for distinguishing these two Aβ pathologies in patients with cognitive impairment.

We also provide new data on other markers that have never been tested in CAA. Neurofilament light is a marker of neuroaxonal damage that has shown great promise as a biomarker in a large number of neurological conditions (343); this includes SVDs (353-355), although age adjusted analyses were only performed in one study (355). We did not find a difference between the AD, CAA and HV groups in age adjusted analyses for either CSF or serum NFL, but in unadjusted analyses the CAA group had higher CSF and serum NFL than the HV group; there was no significant difference between CAA and AD groups. It may be that NFL has greater potential as a longitudinal marker of disease progression (as seems the case in SVD (354) and multiple sclerosis (397-399)) rather than a diagnostic one, particularly as it can be measured in the serum; further longitudinal studies will be needed to investigate this. We also found that CSF ferritin was increased in patients with CAA in unadjusted but not adjusted analyses, compared with both AD and HV groups. Ferritin has promise in CAA as a potential marker for the haemorrhagic manifestations of the condition, particularly for acute convexity subarachnoid haemorrhage and subsequent cortical superficial siderosis. For both NFL and ferritin, the adjusted results may not have reached statistical significance due to our small group sizes, and further evaluation in larger studies will be important. We did not find any differences in sTREM2 between the AD, CAA or control groups; this is in contrast with

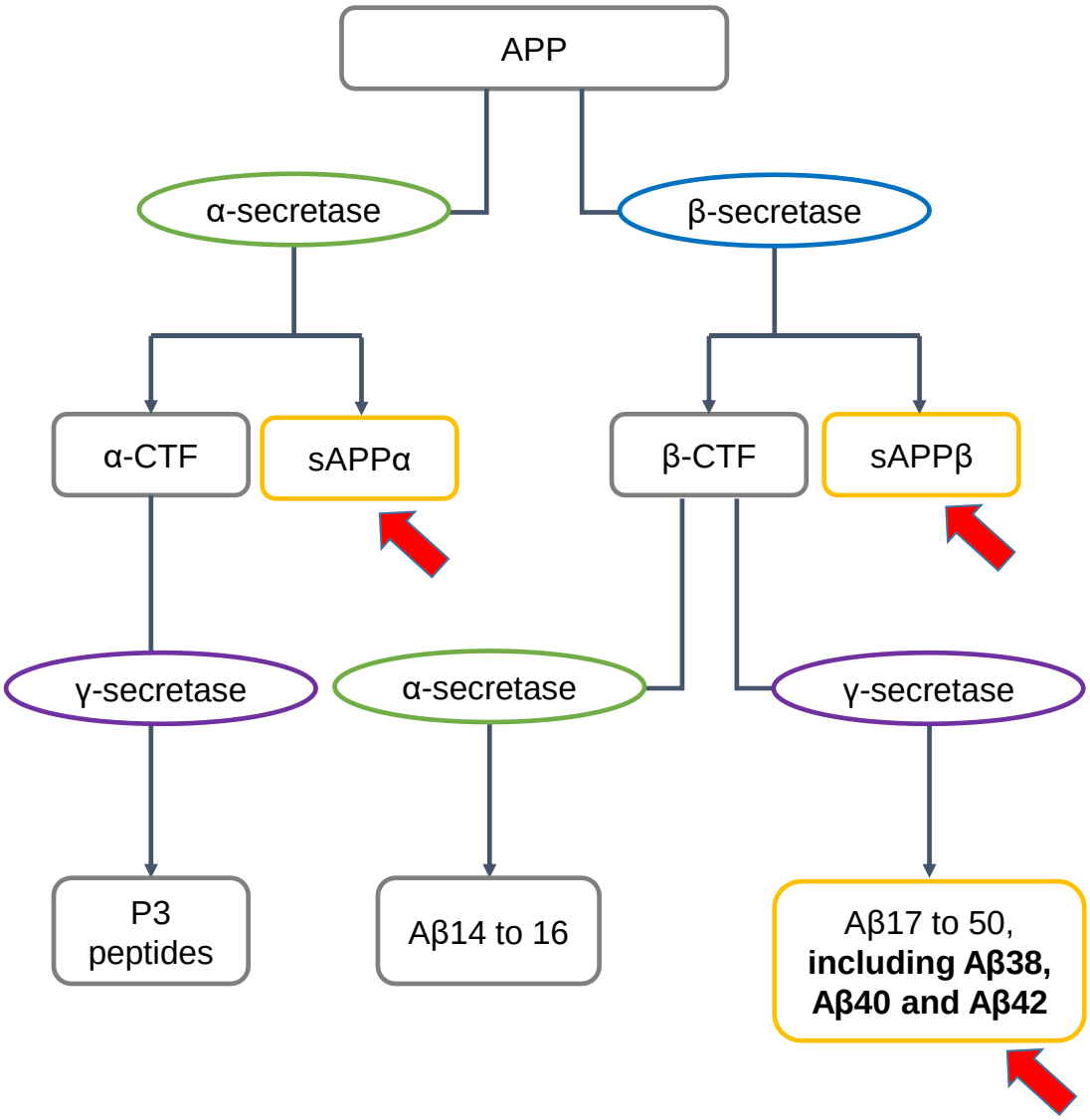
previous studies, in which sTREM2 was found to be elevated in the CSF of AD patients (358-362); again, this may reflect our small sample size.

Our work has a number of strengths. We were able to evaluate a large number of markers and we have used two separate statistical methods, one rank-based (Kruskal-Wallis) and the other an age adjusted analysis based upon comparison of medians. However, there are some limitations. As mentioned earlier, this was a small pilot study which may not have been powered to detect differences for all the biomarkers considered, and for this reason our results should not be considered definitive. We wished to select CAA patients with “early” or mild disease (as discussed in Section 4.3); these patients might have lacked marked biomarker perturbations, which might be more common in those with more severe disease. The AD patients selected from the Specialist Cognitive Disorders Service might not be representative of AD patients more generally; this is a highly specialist tertiary service, which often sees younger patients or those with atypical presentations. Additionally, we screened our healthy volunteers for those with minimal radiological evidence of brain pathology (WMH, atrophy), and this may not be truly representative of age matched individuals without AD or CAA. However, despite these limitations, we provide important new data on these body fluid markers in CAA, and in particular provide data on effect sizes that will be critical for determining sample sizes for larger future studies.

In summary, our findings suggest that patients with CAA appear to have a distinct CSF profile from both patients with AD and age matched controls, characterised by a global reduction in Aβ species, but normal synaptic protein levels (tau, neurogranin) compared with AD patients. Other markers, including CSF NFL, serum NFL and ferritin, showed significant signals in unadjusted analyses. Replication of these findings in larger longitudinal cohorts will be essential for confirming these measures as effective biomarkers for CAA.

Figure 4.5.13: Schematic of pathways for APP processing

Figure adapted from (388); red arrows indicate species measured by our CSF analyses. CTF, C terminal fragment.



4.6 Amyloid-PET

4.6.1 Introduction

Positron emission tomography (PET) is a non-invasive *in vivo* molecular imaging technique that uses targeted radiotracers to quantify biological processes at a molecular level (400). The development of radiotracers that could bind Aβ were primarily developed for the quantification of amyloid pathology in Alzheimer's disease (3); this not only transformed the way in which the disease was diagnosed (and consequently, how patients were selected for therapeutic trials), but also provided new insights into the complex interactions that lead from protein aggregation to clinical phenotype (401). The first amyloid ligand to be used extensively in a research context was the ^{11}Carbon based Pittsburgh-B compound (PiB) (402), but its clinical application was limited by the short half-life of ^{11}Carbon (approximately 20 minutes), which requires the tracer to be synthesised on site using a cyclotron, a type of particle accelerator (403). However, the development of newer ^{18}Fluorine based ligands, which have a much longer half-life (approximately 110 minutes (403)), has resulted in more widespread clinical use of amyloid-PET; three tracers (^{18}F-florbetapir, ^{18}F-florbetaben, ^{18}F-flutemetamol) are now approved by the US Food and Drugs Administration and European Medicines Agency (401). This has resulted in amyloid-PET being more accessible and practically applicable in both research and clinical contexts, making it an exciting new technique for application in CAA.

As described in detail in Section 1.1., CAA is pathologically characterised by the deposition of Aβ protein within the arterioles and capillaries of cortical and leptomeningeal vessels (404). There is a generally accepted consensus that CAA pathology has a posterior predominance, with the greatest burden in parietal and occipital areas (404-411). There is pathological evidence that amyloid-PET tracers are able to bind vascular amyloid (412-414); however, the vast majority of evidence for PET

positivity in CAA comes from studies comparing CAA with other disease groups. The main findings to date are summarised in Table 1.5.1; briefly, amyloid-PET using PiB and ^{18}F-florbetapir binding is able to distinguish between CAA-associated ICH and hypertension (i.e. DPA) associated ICH (111, 112), and regions with higher PiB retention in patients with CAA are associated with an increased risk of subsequent haemorrhage. However, the ability of amyloid-PET to differentiate between CAA patients and either age matched controls or AD patients is less clear. A recent meta-analysis of 7 studies found that amyloid-PET had "moderate to good diagnostic accuracy" for CAA, with overall pooled sensitivity 79% (95% CI 62 to 89%) and specificity 78% (95% CI 67 to 86%) (106), values far lower than the reported sensitivity and specificity of the modified Boston criteria (which are 94.7% and 81.2% respectively (57)). PiB-PET was unable to distinguish patients with CAA from healthy controls in the one study considering this (108), but early uptake, a surrogate for cerebral perfusion, was better able to do this (109). Patients with CAA may have greater occipital PET uptake compared with AD patients, and both the occipital/posterior cingulate ratio (109) and occipital/global ratio (110) have been proposed to differentiate between the AD and CAA. Important questions remain about whether amyloid-PET is a useful diagnostic tool in CAA, given that nearly a quarter of healthy individuals aged over 50 and without dementia have positive amyloid scans (415) and the known overlap between CAA and AD pathology (14). However, amyloid-PET could still be useful as a biomarker for CAA in clinical trials, if it is found to correlate well with disease presence or severity.

In this project, our primary aim was to investigate whether amyloid-PET imaging (using the ligand ^{18}F-florbetapir) would be able to distinguish between cognitively normal patients with "early" haemorrhagic CAA (i.e. a cohort less likely to have coexisting AD pathology), and age matched healthy volunteers. Our second aim was to investigate whether there was any association between amyloid-PET burden and CSF Aβ measures in our participants.

4.6.2 Methods

4.6.2.1 Patient selection

The selection criteria and recruitment methods for the BOCAA study are described in Sections 4.2 and 4.3. We included 10 patients with CAA, and 5 age matched healthy volunteers.

4.6.2.2 PET acquisition and processing

These methods were provided by Dave Cash (affiliated with the Dementia Research Centre, and Centre for Medical Imaging Computing, UCL). Details for the imaging acquisition are taken from reference (336).

All PET and MR imaging data was acquired on a single Siemens Biograph PET/MR scanner (336). The amyloid-PET ligand ^{18}F-Florbetapir ("Amyvid", produced by Eli Lilly) was injected via a peripheral cannula (activity 370MBq), with continuous acquisition from the start of the injection (336).

Static PET images representing uptake of ^{18}F-Florbetapir tracer 50 to 60 minutes post-injection were reconstructed using a pseudo CT method for attenuation correction (416). This method has been thoroughly validated in many data sets across multiple sites and scanner models, producing robust and accurate attenuation correction compared to a gold standard of an actual CT scan (417). Standard Uptake Value Ratio (SUVR) images with and without partial volume correction (PVC) were produced (418); this BOCAA analysis used images without PVC. The resulting post-uptake was then rigidly registered to the structural MRI scan using a symmetric block matching technique (419). MR scans were parcellated using the Geodesic Information Flow (GIF) algorithm (420), and all voxels in the post-uptake image were then normalised to a reference region to produce

an SUVR image. The primary reference region of choice was a mask of subcortical white matter, eroded one time to avoid partial volume effects, but SUVR images with a whole cerebellar reference region were also produced as an alternative; the BOCAA analysis used SUVR images calculated form the cerebellar reference region, as the impact of the severe white matter damage observed in the CAA patients on uptake was uncertain. A global measure of amyloid burden was computed using SUVR from a composite cortical region of interest (ROI), based on a weighted (by volume) average of GIF regions. This composite ROI was chosen to match as closely as possible to the FreeSurfer-based composite ROI used in ADNI (421).

4.6.2.3 Thresholds for amyloid positivity

Initial visual reads were performed by a trained rater (John Dickson, Institute of Nuclear Medicine, UCLH). The following methods were provided by Dave Cash (affiliated with the Dementia Research Centre, and Centre for Medical Imaging Computing, UCL).

The threshold for amyloid positivity was determined from the Insight 46 study (a large birth cohort study), of which 451 individuals (aged 69 to 71) had suitable MR and PET for the amyloid SUVR processing pipeline. Gaussian mixture models were used to fit the data and obtain a threshold for positivity. We tested mixture models with one, two, and three Gaussians, with the best model selected using Bayesian Information Criteria. The best fit for the composite cortical ROI was two Gaussians. One of the Gaussians had a much larger mixing proportion that had a lower mean and lower variance. This represented the large number of individuals in the cohort who were normal and showed no evidence of amyloid deposition. The other distribution had a lower mixing proportion, a higher mean and much larger variance. This represented the amyloid positive individuals (Figures 4.6.1 and 4.6.2). Given the wider variance and limited number of subjects in the latter Gaussian, we chose a threshold based on the 99th percentile from

the amyloid negative distribution as a cut-off. The resulting threshold for non-PVC corrected data was 0.61 for a subcortical white matter reference and 1.078 for a whole cerebellar reference. The cut-off for the whole cerebellar reference region closely corresponds to the value of 1.10 that is frequently used in studies with this tracer (422, 423).

4.6.2.4 Calculation of regional SUVRs

Each GIF parcellation was visually inspected by the candidate for errors. Errors that were identified included labelling of extracerebral regions as cortical (Figure 4.6.3) and mislabelling of the cerebellum (Figure 4.6.4). In the patient group, the presence of brain pathology also resulted in parcellation errors, particularly white matter damage (Figure 4.6.5) and areas previously affected by ICH (Figure 4.6.6). Cortical areas that were incorrectly labelled were excluded from the analysis. If the cerebellum was grossly misidentified, SUV data for the participant was excluded from the analysis. Regional SUVRs were calculated by summing values for mean SUVR multiplied by volume for each subregion, and then dividing by the total volume for that region i.e. Σ (mean SUVR*volume) for all sub-regions / total regional volume. “Global” uptake was defined as the total grey matter cortical uptake, and included all listed cortical areas. A region was defined as positive if the uptake was greater than the regional threshold specified for that area.

4.6.2.5 Statistics

Statistical analysis was performed by the candidate using Stata (Version 15.1). Global and regional ^{18}F-florbetapir SUVRs for patients and healthy volunteers were compared using the (non-parametric) Mann Whitney U test. Chi-squared tests were used to compare global and regional positivity for patients with CAA and healthy volunteers. For comparisons between PET positive and PET negative patients, independent t-tests were used for continuous data (age, years of education), the Mann Whitney U for variables that were continuous but not normally distributed (mRS, MMSE, MoCA, WMH grade, MTA and GCA grade, lobar CMB, CSF measures) and chi-squared for all categorical variables. Multivariable adjusted comparisons were not performed due to the small sample size.

Figure 4.6.1: Histogram of SUVR values with subcortical white matter as reference region

Figure produced and provided by Dave Cash, Dementia Research Centre and Centre for Medical Imaging Computing, UCL

Overlaid on the histogram are the individuals' Gaussians and combined distribution coming from a Gaussian mixture model. Defining the cut-off as the 99th percentile of the amyloid negative distribution gives a result of 0.61.

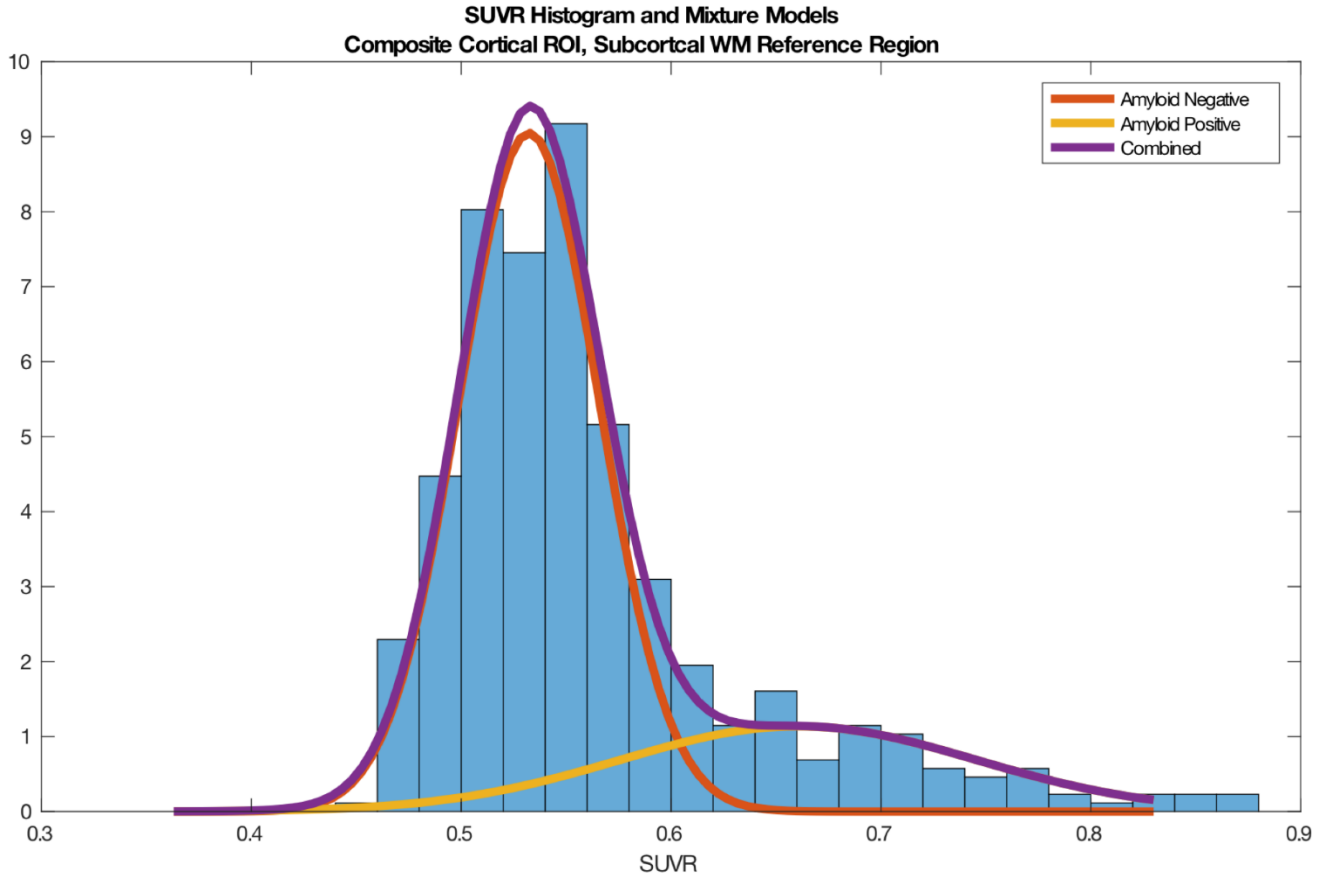


Figure 4.6.2: Histogram of SUVR values with whole cerebellum as reference region

Figure produced and provided by Dave Cash, Dementia Research Centre and Centre for Medical Imaging Computing, UCL.

Overlaid on the histogram are the individuals' Gaussians and combined distribution coming from a Gaussian mixture model. Defining the cut-off as the 99th percentile of the amyloid negative

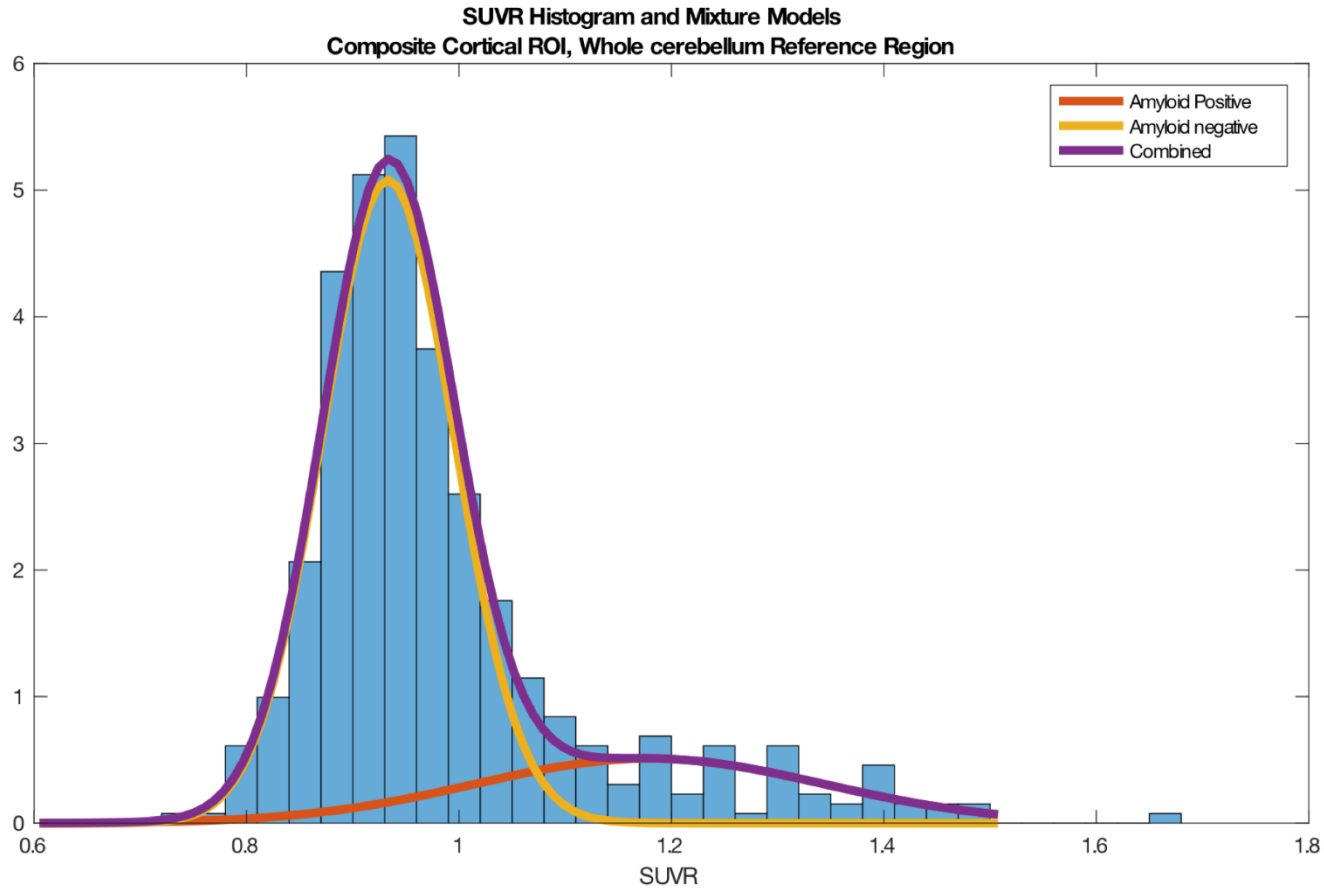


Figure 4.6.3: Examples of incorrect labelling of extracerebral regions by the GIF parcellation algorithm

These images show GIF parcellation labels overlaid onto T1 images. Panel A shows mislabelling of dura as cortical regions (arrows); panel B shows extracranial regions incorrectly labelled as temporal lobe (arrows).

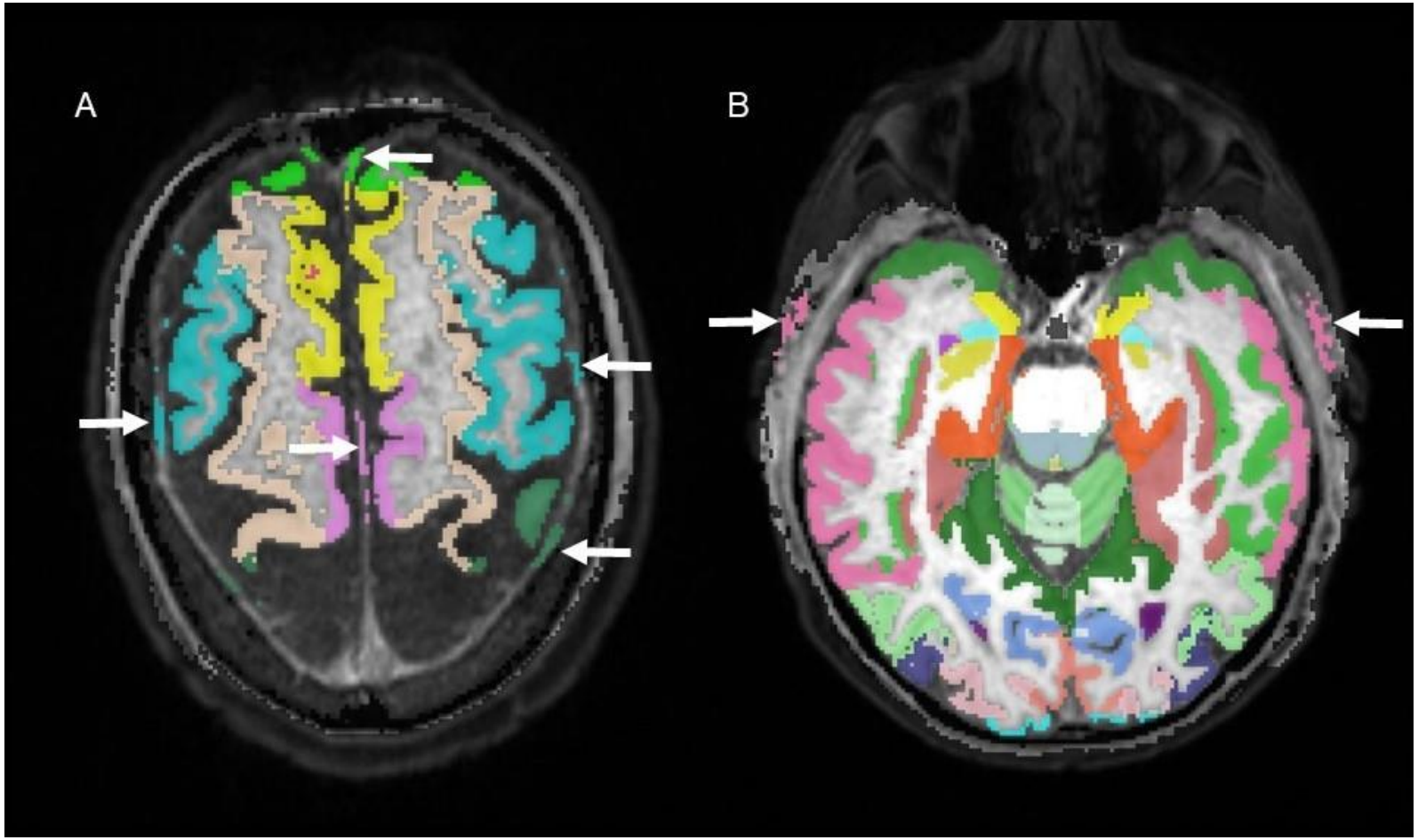


Figure 4.6.4: Examples of incorrect GIF parcellation of the cerebellum

These images show GIF parcellation labels overlaid onto T1 images. Panel A shows extracranial regions incorrectly labelled as cerebellum; in panel B, a large area of temporal lobe on the right has been incorrectly labelled as cerebellum.

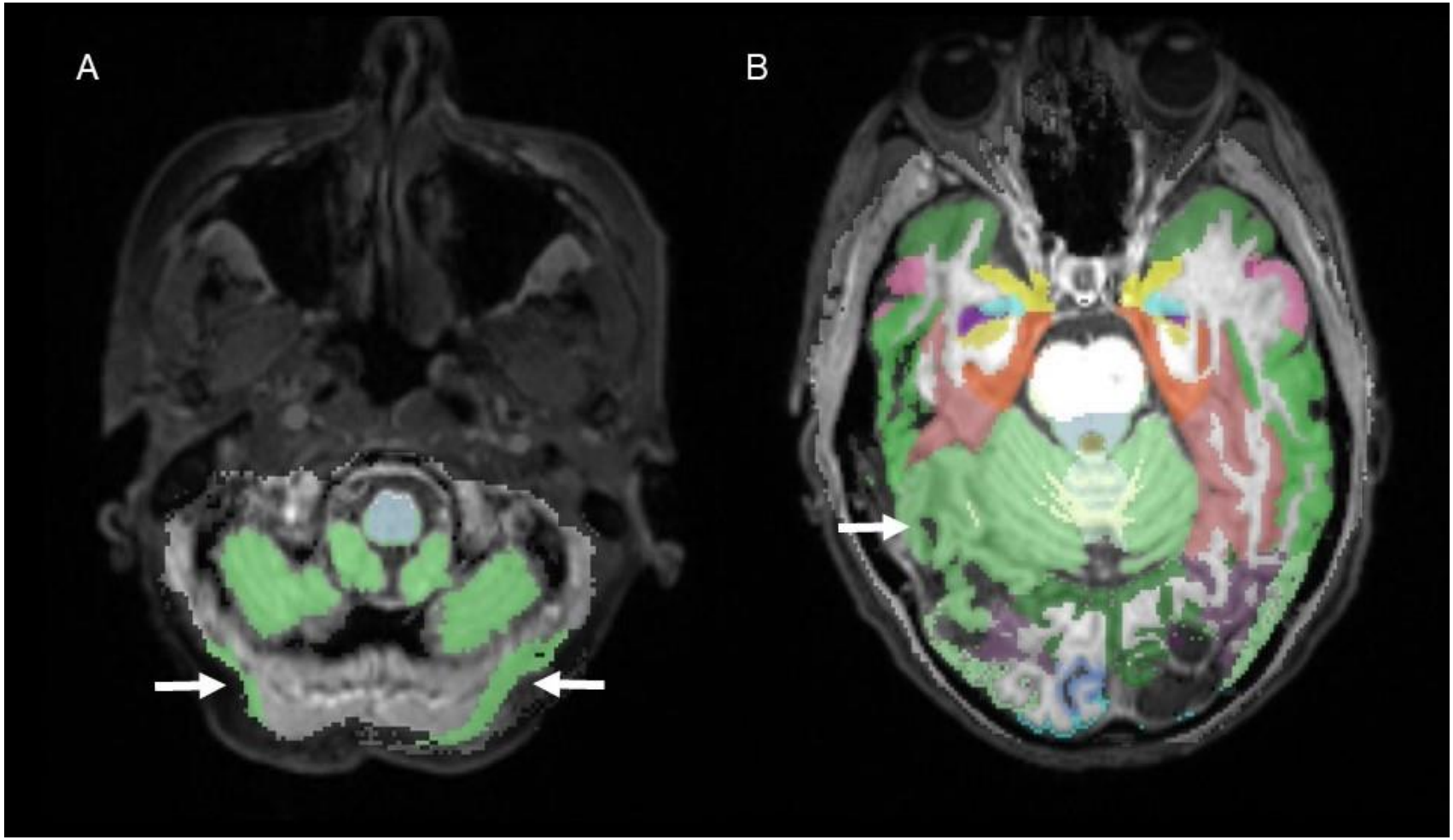


Figure 4.6.5: Examples of mislabelling of white matter damage by the GIF parcellation algorithm

Figure shows GIF parcellation labels overlaid onto T1 images on the left (A, C), and corresponding T1 without labels on the right (B, D). In panel A, a large area of white matter damage has been incorrectly identified as cortex (arrow); panel C shows a smaller region mislabelled in a similar way (arrow).

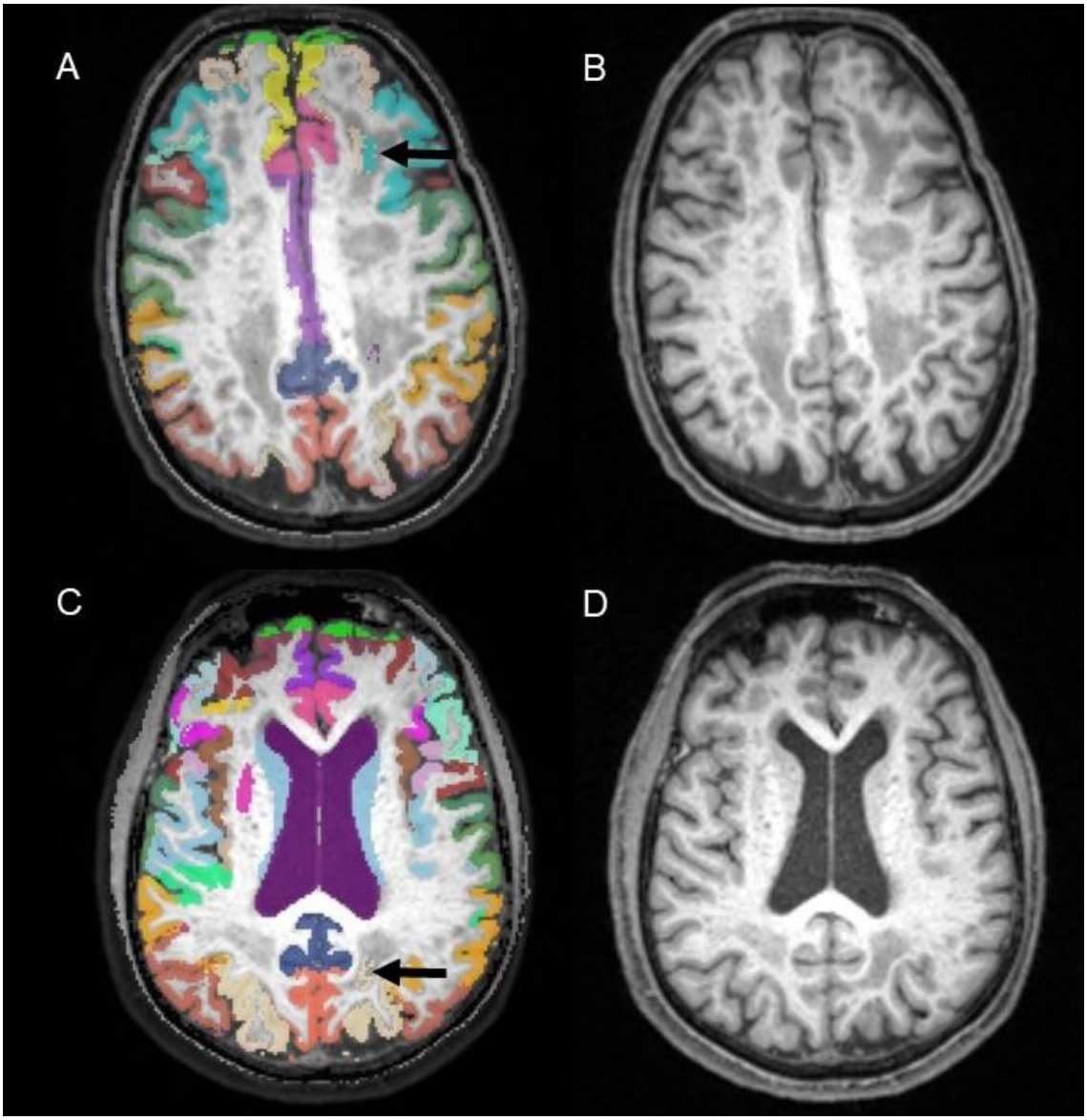
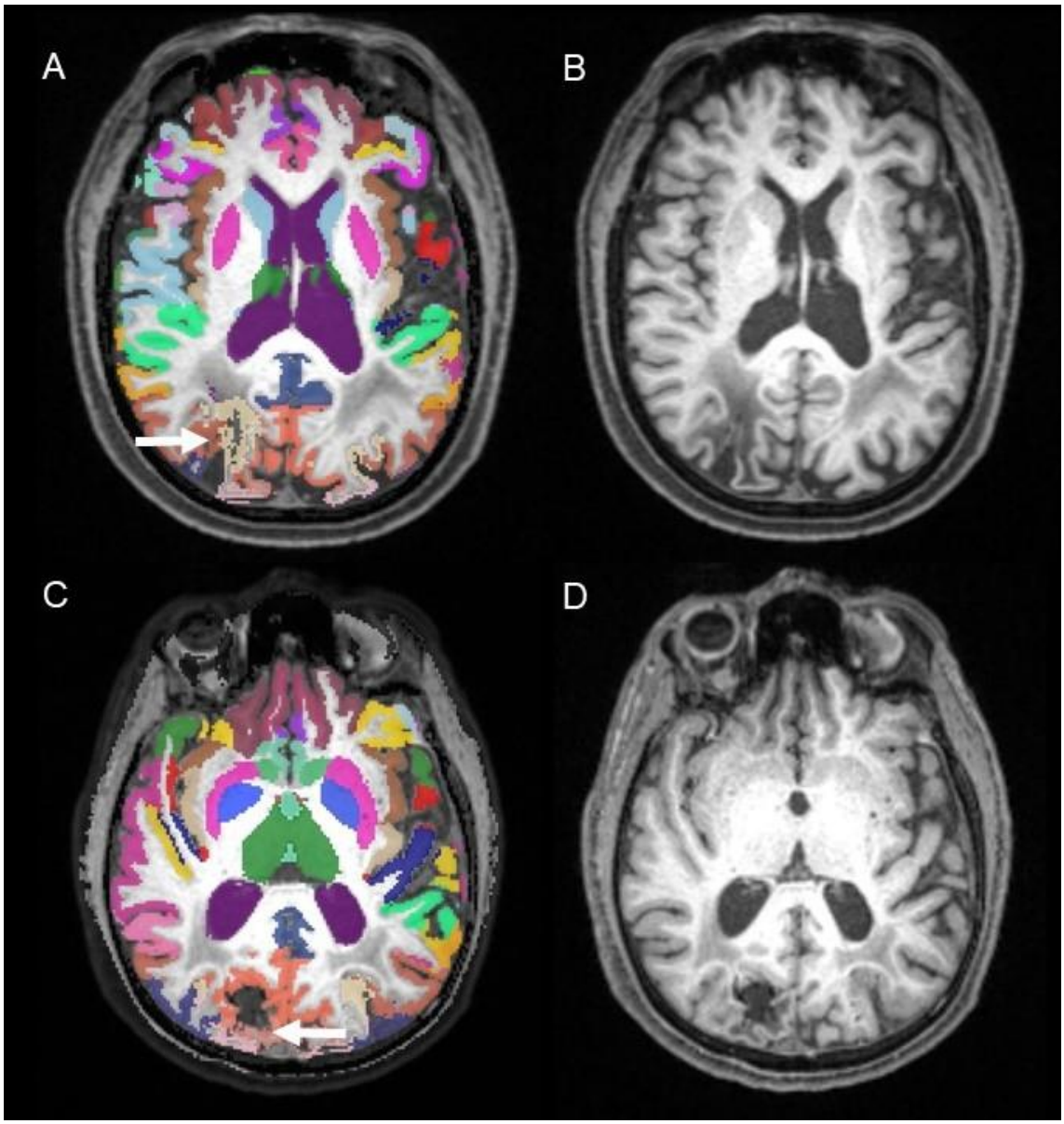


Figure 4.6.6: Examples of mislabelling of areas affected by previous ICH by the GIF parcellation algorithm

Figure shows GIF parcellation labels overlaid onto T1 images on the left (A, C), and corresponding T1 without labels on the right (B, D). Panels A and C show areas damaged by previous haemorrhage being incorrectly labelled as cortex (arrows).



4.6.3 Results

The baseline characteristics of the BOCAA participants have been described (Section 4.4); there were no statistically significant differences in baseline clinical and demographic characteristics between the patient and healthy volunteer groups. According to the initial visual reads, 5 of the patients with CAA were PET positive, compared with none of the healthy volunteers (50% vs 0%, one-sided Fisher's exact p=0.084).

One patient was excluded from the SUVR analysis due to incorrect segmentation of their cerebellum (Figure 4.6.4, panel B). There were no significant differences in global or regional SUVR values between patients and healthy volunteers (Table 4.6.1). When comparing global and regional PET positivity, as defined by standardised SUVR thresholds (Table 4.6.2), again there were no statistically significant differences between the two groups. A higher proportion of patients with CAA showed PET positivity in the occipital lobe and precuneus than in the control group, but this did not reach statistical significance (Table 4.6.2).

When comparing participants who were PET positive (i.e. those who had global cortical uptake greater than the standardised threshold) with those who were PET negative (Table 4.6.3), there were important but statistically non-significant differences in age (PET positive patients were older, 70.0 years vs 65.3 years, p=0.0684) and history of migraine with aura (PET positive patients 33% vs PET negative 87.5%, p=0.091). PET positive patients were more likely to have cSS (83.3% vs 25.0%, p=0.044) and had lower levels of CSF Aβ-42 (median 115.0 pg/ml vs 224.0 pg/ml, p=0.0389).

Table 4.6.1: Global and regional ^{18}F-florbetapir PET SUVR values for the BOCAA study

P values are from Mann Whitney U tests.

	CAA patients	Healthy Volunteers	p value
n	9	5	-
Global SUVR, median (IQR)	1.11 (1.01 to 1.17)	0.94 (0.94 to 1.03)	0.4634
Frontal SUVR, median (IQR)	1.11 (0.98 to 1.18)	0.95 (0.94 to 1.05)	0.5045
Parietal SUVR, median (IQR)	1.07 (1.00 to 1.17)	0.91 (0.91 to 1.00)	0.2856
Occipital SUVR, median (IQR)	1.09 (1.01 to 1.20)	0.94 (0.93 to 1.01)	0.2053
Temporal SUVR, median (IQR)	1.09 (0.96 to 1.14)	0.94 (0.94 to 1.00)	0.3173
Anterior and middle cingulate SUVR, median (IQR)	1.14 (1.13 to 1.30)	1.05 (1.04 to 1.13)	0.5045
Posterior cingulate SUVR, median (IQR)	1.16 (1.06 to 1.24)	1.04 (1.01 to 1.06)	0.4232
Precuneus SUVR, median (IQR)	1.19 (1.06 to 1.27)	0.94 (0.93 to 0.95)	0.0231

Table 4.6.2: Global and regional ^{18}F-florbetapir PET positivity, using standardised SUVR thresholds

P values are from one-sided Fisher's exact tests.

	Threshold for positivity, SUVR	CAA patients	Healthy Volunteers	p value
n	-	9	5	-
Global positivity (all cortical grey matter), n (%)	1.072	5 (55.6)	1 (20.0)	0.238
Frontal positivity, n (%)	1.075	5 (55.6)	1 (20.0)	0.238
Parietal positivity, n (%)	1.070	5 (55.6)	1 (20.0)	0.238
Occipital positivity, n (%)	1.072	6 (66.7)	1 (20.0)	0.133
Temporal positivity, n (%)	1.059	5 (55.6)	1 (20.0)	0.238
Anterior and middle cingulate positivity, n (%)	1.165	4 (44.4)	1 (20.0)	0.378
Posterior cingulate positivity, n (%)	1.150	5 (55.6)	1 (20.0)	0.238
Precuneus positivity *(CAA patients n=8)*	1.066	6 (75.0)	1 (20.0)	0.086

Table 4.6.3: Comparison of PET positive and negative patients

P values are from t-tests (where mean and SD given), Mann Whitney U tests (where median and IQR given), or two-sided Fisher's exact tests (remainder).

		PET positive	PET negative	p value
n		6 (42.9)	8 (57.1)	-
Age, years, mean (SD)		70.0 (2.8)	65.3 (5.3)	0.0684
Sex, female, n (%)		2 (33.3)	3 (37.5)	1.000
Past Medical History				
Hypertension, n (%)		4 (66.7)	4 (50.0)	0.627
Hypercholesterolaemia, n (%)		4 (66.7)	2 (25.0)	0.277
Diabetes mellitus, n (%)		1 (16.7)	0 (0.0)	0.429
Seizures, n (%)		1 (16.7)	0 (0.0)	0.429
Migraine with aura, n (%)		2 (33.3)	7 (87.5)	0.091
TFNE, n (%)		2 (33.3)	2 (25.0)	1.000
Previous ICH, n (%)		2 (33.3)	2 (25.0)	1.000
Years of education, mean (SD)		17.8 (3.7)	20.1 (2.7)	0.2040
mRS, median (IQR)		0 (0 to 0)	0 (0 to 0)	0.3865
MMSE score, median (IQR)		29 (29 to 30)	29.5 (28.5 to 30)	0.6792
MoCA score, median (IQR)		27.5 (26 to 29)	27 (25 to 28.5)	0.6455
WMH score, median (IQR)	dWMH	1 (1 to 1)	1 (0 to 2)	0.7257
	pvWMH	2.5 (2 to 3)	1.5 (1 to 3)	0.3387
Lacunes, n (%)		1 (16.7)	0 (0.0)	0.429
MTA grade, median (IQR)		1 (1 to 1)	1 (0 to 1)	0.2519
GCA grade, median (IQR)		1 (1 to 2)	1 (0 to 2)	0.5887
cSS, n (%)	None	1 (16.7)	6 (75.0)	0.044
	Focal	3 (50.0)	0 (0.0)	
	Disseminated	2 (33.3)	2 (25.0)	
Lobar CMBs, median (IQR)		2 (1 to 17)	0 (0 to 3.5)	0.1996
CSF Aβ-38, pg/ml, median (IQR)		2476.8 (1434.5 to 2613.5)	1638.5 (1485.5 to 2068.8)	0.4386
CSF Aβ-40, pg/ml, median (IQR)		4253.3 (3169.0 to 4445.0)	3440.8 (3085.0 to 4629.0)	0.6985
CSF Aβ-42, pg/ml, median (IQR)		115.0 (92.5 to 134)	224.0 (135.3 to 341.0)	0.0389
CSF sAPPα, pg/ml, median (IQR)		90.5 (67.9 to 103.0)	90.2 (72.1 to 135.0)	0.6056
CSF sAPPβ, pg/ml, median (IQR)		85.8 (79.0 to 108.0)	91.7 (74.9 to 139.8)	0.5186

4.6.4 Discussion

Our main findings were firstly, that amyloid-PET was not a useful discriminator between CAA patients and healthy volunteers, with only half of our CAA patients meeting the threshold for PET positivity. Secondly, when we compared participants with positive and negative PET scans, we found that those with positive scans were more likely to have cSS and lower levels of CSF Aβ-42. Finally, we encountered significant technical difficulties with the automatic segmentation algorithm used, in particular for our patient group. Automated image processing pipelines are potentially hugely advantageous as they allow for rapid processing of large datasets in a standardised way, and it will be crucial for the issues identified by this pilot to be solved, in order for these automated methods be reliably applied to patients with significant brain pathology.

We found no differences in amyloid-PET signal between our patient and healthy volunteer groups using either continuous SUVR values or standardised thresholds for positivity. Additionally, we did not find that PET positivity was associated with cognitive scores (MMSE, MoCA), or clinical features of CAA (history of TFNE or symptomatic parenchymal ICH). Whilst our results must be interpreted with caution due to the errors in cortical segmentation and a potential lack of statistical power, they are in in keeping with previously published work (108), which found that late amyloid-PET uptake was poor at discriminating between CAA patients and age matched controls. There is data suggesting that early amyloid-PET tracer uptake is impaired in CAA (109), and thus legitimate questions remain about whether the tracer is able to adequately reach the relevant cerebrovascular Aβ pathology. Additional concerns are the relatively high prevalence of “asymptomatic” amyloid in healthy volunteers (our rate of 20% is not dissimilar to previously reported estimates (415)), and the known overlap between AD and CAA (14), which raises questions about whether amyloid uptake in CAA is actually labelling parenchymal rather than vascular deposits (110). Finally, lessons learnt from therapeutic trials in AD might also have relevance for CAA (424, 425); this includes the

fact that amyloid burden may be within normal limits in up to 30% of patients with clinically diagnosed AD, and that amyloid load might be a marker of relatively late-stage disease (424). Whether amyloid-PET is useful longitudinally in CAA remains to be seen.

Our finding that PET positivity was associated with cSS is in keeping with previously published work (72, 95, 426). Interestingly, cSS is also associated with cognitive impairment (32, 72, 123, 159, 427-431), and has been associated with both the *APOE* ε4 allele and a pattern of cortical atrophy (involving the precuneus, posterior cingulate, parietotemporal, superior frontal, and medial temporal cortices) similar to that seen in AD (95). We also found that CSF Aβ-42 was lower in patients with positive PET scans, another finding typically associated with AD (383). Thus our findings might be interpreted as evidence that in patients with CAA, amyloid-PET positivity is reflective of co-existent AD-like pathology; replication of these findings in larger cohorts will be needed to confirm this.

The strengths of this work are the detailed phenotyping available for each patient, our use of a standardised research and imaging protocol for all participants, our ability to measure amyloid burden using two modalities (PET and CSF), and the threshold values, which have been obtained from a large number of participants. The limitations are firstly, our small sample size, which means that our negative results are not definitive and might simply reflect a lack of power. We did not include data on early uptake in this work, which might have provided a mechanism for why such a large proportion of our CAA patients were PET negative (i.e. due to tracer uptake issues); this is an area for future work. The difficulties with the automated parcellation method have been described (Section 4.6.2.4) and this could have resulted in artificially low SUVR values in the patient group, who had more brain pathology and thus more errors. The next stage of this project will be to improve these parcellations, through a combination of lesion masking, identification and exclusion of WMH using FLAIR sequences, and additional processing to remove the

extracranial labelling. One potential limitation of PET in CAA patients with previous ICH is that the location of their previous haemorrhage(s) might have been the site of greatest amyloid uptake (based on the hypothesis that amyloid pathology increases bleeding risk). However, these areas can never be assessed once they are damaged by the ICH, and so the uptake observed might be artificially low. Finally, we chose to use the cerebellum as our reference region, a region which can be affected by vascular amyloid (432, 433); other regions, for example the pons, might be for reliable for CAA.

To summarise, in this small pilot study we did not find that amyloid-PET using ^{18}F-florbetapir was able to distinguish patients with CAA from healthy volunteers; PET positivity was not associated with cognitive performance or clinical markers of CAA, but there were associations with the presence of cSS and lower levels of CSF Aβ-42. Although our results have significant limitations, they support the argument that amyloid-PET might be of limited use as a biomarker for therapeutic clinic trials; it could be used as a selection criteria for future anti-amyloid strategies in CAA (in a manner akin to that used in AD), but further work on whether it can detect all presentations of CAA and whether it varies longitudinally is needed.

5 Discussion

The three main aims of the programme of research described in this PhD thesis were described in Section 1; this discussion will now consider how the results presented here were able to address these three objectives, before highlighting outstanding questions, suggesting directions for future research, and drawing final conclusions.

5.1 What is the role of SVDs and their neuroimaging markers in different patient populations?

This thesis contains data from four projects which aimed to address this question. Together, these results demonstrate that structural markers of SVD have relevance in a diverse range of patient populations.

The main findings were:

1. In a memory clinic population, CSO-PVS were associated with ADCI, whereas BG-PVS were associated with SVCI; however, CSO-PVS was not independently associated with PiB positivity. This work provides further supporting evidence that CSO-PVS are a key imaging marker for AD, and raises the possibility that CSO-PVS are a measure of vascular amyloid processes that are not identified by amyloid-PET, amyloid independent processes, or both.

2. In patients presenting with spontaneous ("primary") ICH, the presence of cognitive impairment prior to the index ICH event was associated with imaging markers of CAA (fulfilling the modified Boston criteria for probable CAA, and per point increase of a composite CAA score). This finding shows that haematoma damage cannot be the only mechanism contributing to cognitive disruption in patients with CAA, and supports the hypothesis that small vessel mechanisms are important for this disruption.

3. In patients with AF-associated ischaemic stroke and TIA, we found that nearly a quarter of patients (24.7%) met IQCODE criteria for pre-existing cognitive impairment. Pre-existing cognitive impairment was associated with the presence of lacunes, pvWMH, dWMH, and MTA (but not with other structural markers of small vessel disease), and was also associated with both acute post-event cognitive performance and functional outcome at 24 months.

4. When comparing acute (immediate post-event) MoCA performance and 12 month MoCA performance in patients with AF-related ischaemic events, we found that, overall, performance at 12 months was improved. When comparing "reverters" (patients with an acute MoCA score <26, with improvement of ≥2 points at 12 months) and "non-reverters" (those with acute MoCA score <26 who did not show this improvement), we found that the presence of structural imaging markers of small vessel disease (CSO-PVS, CMBs, composite SVD and CAA scores) is associated with non-reversion.

In answer to the question "What is the role of SVDs and their neuroimaging markers in different patient populations?" we can say firstly that the presence of these markers is common across the three populations considered (those attending a memory clinic, patients presenting with ICH, and those with AF-related ischaemic events). We also provide evidence that these markers are associated with clinical measures, including diagnosis (project 1; Section 2.4) or cognitive measures (remainder). Finally, this work shows that, on the whole, no single SVD subtype is responsible for the phenotype observed; the possible exception to this (from the work described in this thesis) is the impact of CAA in patients with ICH (project 2; Section 2.5). This is in keeping with data from neuropathological data from population-based studies of the elderly, which show that multiple different neurodegenerative and cerebrovascular pathologies interact and

contribute to the eventual cognitive phenotype (126, 434, 435). There are two further conclusions that can be drawn from the results presented. Firstly, cerebrovascular pathology is likely to play a role in conditions considered “neurodegenerative”; this is one interpretation of the results presented in project 1 (Section 2.4), and is further supported by neuropathological work which shows CAA has an independent contribution to AD diagnosis (50). Secondly, in patients presenting with “cerebrovascular” diagnoses (i.e. stroke, either haemorrhagic or ischaemic), SVD pathologies are likely to interact with each other and with neurodegenerative processes; to consider cognitive impairment in this context as purely “vascular” is likely to be a gross oversimplification.

It is important to recognise and acknowledge the limitations of MR-based structural markers of SVD. Firstly, these methods are only semi-quantitative and thus partially subjective, which can result in variation between raters. Attempts were made to reduce the impact of this by ensuring that a given imaging feature was rated by a single individual where possible, and when this was not the case, using kappa values to confirm adequate interrater reliability. Whilst this limitation can be avoided by using purely quantitative analysis methods, they are not available for all structural markers. Another limitation of these structural imaging markers is that they can only be used in patients with MRI data, which almost certainly results in a biased study population, with a bias towards including patients with milder disease (i.e. those able to tolerate an MRI) and without certain comorbidities (e.g. those with pacemakers). We tried to address this issue by comparing included and excluded participants, but inevitably complete quantification of this is not possible.

Although the data described here are observational, making it impossible to draw conclusions about causality, the evidence suggests that SVDs are present and appear to contribute to cognitive phenotype in three independent patient populations. This might suggest that pure “neurodegenerative” or “vascular” cognitive impairment is unusual, and

thus a wide range of cognitive syndromes may be amenable to future strategies that aim to mitigate SVD related damage.

5.2 How does SVD subtype and burden influence clinical outcomes in patients with ICH?

This thesis presents the results from two projects that aim to address this question, using data from the CROMIS-2 ICH study, which included patients presenting with spontaneous ICH. The main findings are:

1. At 3 year follow up, overall there were fewer ICH events than cerebral ischaemic events (45 vs 70), but the opposite was observed in the lobar ICH group, where there were more ICH events (n=35) than cerebral ischaemic events (n=29). Lobar ICH location was independently associated with a higher risk of recurrent ICH events, but there was no association between ICH location and the risk of subsequent cerebral ischaemic events. In addition to ICH location, recurrent ICH events were associated with a history of previous ischaemic events, antiplatelet use prior to study entry, and increasing Van Swieten score, whereas the occurrence of subsequent cerebral ischaemic events were associated with increasing age, AF, and a history of previous ischaemic events.

2. Death within 3 years of ICH was independently associated with age at study entry, smoking, pre-event mRS and NIHSS at presentation, but not ICH location or WMH burden. Death within 6 months of ICH was associated with different factors compared with death after this time, but ICH location and WMH burden were not associated with death during either period. In further exploratory analyses where the time-varying effect of each variable was allowed to vary continuously with time, a history of previous cerebral ischaemic events, initial GCS, NIHSS, Van Swieten score and ICH volume had significant time-varying effects, with the hazard ratios of previous

cerebral ischaemic events and Van Swieten score increasing with time, whilst those for GCS, NIHSS and ICH volume decreased with time.

As discussed in the Introduction (Section 1.2), one of the most important reasons for being able to accurately differentiate between SVD subtypes in life is in patients with ICH, where CAA and DPA are believed to confer different ischaemic and haemorrhagic risk. However, both these projects highlight the limitations of using CT-based imaging to establish SVD subtype.

We found a strong association between lobar ICH location and ICH recurrence, but no association between ICH location and subsequent cerebral ischaemic risk; this could be interpreted as supportive of previous data suggesting that CAA is associated with an increased recurrent ICH risk (15). However, caution is needed as lobar ICH might not just be a surrogate marker of CAA. As described earlier, lobar ICH can occur in the context of hypertension or “mixed” CAA and DPA disease. The authors responsible for the CT-based Edinburgh criteria for CAA found in their cohort patients with lobar ICH, 26 of 62 (41.9%) patients had absent or mild CAA, and of those with lobar ICH with moderate or severe CAA, the majority (26 of 36, 72.2%) had co-existing DPA (58). Moreover, 6 of 48 patients presenting with non-lobar ICH (12.5%) had moderate or severe CAA. These data show the limitations of defining SVD subtype on the basis of ICH location alone. Whilst ICH recurrence was also associated with higher Van Swieten scores, WMH are not specific for SVD subtype. Thus it is not possible to exclude the possibility that a feature specific to lobar ICH but unrelated to CAA is responsible for the recurrent ICH associations observed (for example, that lobar ICH is associated with more severe “mixed” SVD burden).

Our analyses of death did not find direct associations between surrogate markers of SVD (ICH location, Van Swieten score). However, the interpretation of ICH location in the context of death is difficult, as infratentorial haemorrhages (those involving the brainstem and cerebellum) increase the risk of complications such as obstructive hydrocephalus and brainstem compression, are thus are associated with death via mechanisms independent of SVD (318, 319). We did find in our exploratory univariate time-varying analyses that the HR associated with Van Swieten score and death increased with time, but this needs to be interpreted with caution as this analysis was not adjusted for age. The results of these analyses show that the factors influencing death, particularly in the longer term, are complex.

5.3 Are we able to identify new biomarkers for CAA using body fluid measures and amyloid-PET imaging?

This thesis presents initial results from BOCAA, a prospective observational feasibility study designed in order to guide future therapeutic studies in CAA. As well as presenting our recruitment experience and potential pitfalls for larger studies aiming to identify CAA patients for trials, the following preliminary results were presented:

1. We found that patients with CAA had a distinctive body fluid profile, compared to HV and AD groups. In unadjusted analyses, patients with CAA showed lower levels of all amyloid components measured (Aβ-38, Aβ-40, Aβ-42, sAPPα and sAPPβ), and higher levels of serum NFL and ferritin than both other groups. Patients with AD had higher total tau and phospho-tau than both HV and CAA groups; CSF NFL was increased in both CAA and AD groups relative to the HV group, and neurogranin was higher in patients with AD than HV. In age adjusted analyses, differences for the CAA group remained for Aβ-38, Aβ-40, Aβ-42, and sAPPβ; for the AD group, Aβ-42 was significantly different from HV (but not the CAA group).

2. Amyloid-PET was not a useful discriminator between CAA patients and healthy volunteers, with only half of our CAA patients meeting the threshold for PET positivity. Participants with positive amyloid-PET scans were more likely to have cSS and had lower levels of CSF Aβ-42.

Here, we used two different modalities to identify biomarkers for CAA. Although our sample sizes are small, with only 10 CAA patients included in the analyses, we found striking differences in the CSF profile between CAA patients, AD patients and the HV group, which might suggest that this is the biomarker modality with the best discriminative capacity. Our negative findings need to be interpreted with caution as we might have been underpowered to identify smaller effects. Additionally, the PET analyses were complicated by significant technical issues with the GIF parcellation. Whilst this initial data shows promise, it is important to recognise that CAA can present heterogeneously (with cognitive symptoms, TFNE, ICH, or a combination thereof), and this study focused on haemorrhagic, non-cognitive presentations; biomarker profiles might not be uniform across these presentations. Future studies will need to consider this when planning their approach to novel biomarker identification.

5.4 Future directions

The projects described in this thesis have highlighted a number of areas for future research. The limitations of using structural markers of SVD have been discussed, some of which can be addressed through automated volume measurements (in particular, for brain volumes, WMH and PVS). At present, neuropathological studies remain the gold standard for establishing how different cerebrovascular and neurodegenerative pathologies interact in order to contribute to clinical phenotype, but the use of multimodal imaging (for example, combining amyloid and tau PET with MRI) could allow these interactions to be better identified *in vivo*. In addition to this, definitions are likely to

change; the clinical heterogeneity observed in patients with imaging features of CAA, raises questions about whether these different presentations (in particular, cognitive CAA and haemorrhagic CAA) can really be grouped together as a single disease. There is also greater awareness that “pure” CAA in ICH, existing in isolation as the sole contributing neuropathology, is likely to be unusual (58). New methods of identifying DPA will be important for establishing how it influences clinical presentation in the increasing recognised “mixed” phenotype.

This work on outcomes following spontaneous ICH highlights the limitations of using CT to accurately diagnose SVDs, and the difficulties resulting from the low number of cerebrovascular events, in our case despite a large cohort size (over 1000 patients) and lengthy follow up (3 years). The development of new CT-based diagnostic criteria for CAA (58) may allow the first of these limitations to be overcome, and the correlation of these Edinburgh criteria with clinical (rather than neuropathological) outcomes will be crucial for their validation. Further studies which follow a similar or larger number of participants for longer time periods (at least 5 years, up to 10 years), ideally with MRI for all participants at baseline, would allow for the accrual of more events; this will be important for attaining more meaningful and robust conclusions regarding long-term cerebrovascular outcomes in these patients. Interval assessments of these patients, where imaging (again, ideally MRI) and assessment of clinical factors (for example, medication use, cognitive performance, and changes to baseline measures such as smoking) are repeated, would again allow for a more detailed understanding of outcomes in these patients, and would be particularly useful for further work into how time-varying effects influence outcome. Finally, all observational studies in ICH are likely to be biased towards a “survivor” population, and research which includes all patients presenting with an acute ICH to stroke services is needed to account for this.

A large amount of data from the BOCAA study is yet to be analysed, and there is great potential for new biomarkers to be identified from this work. This includes analysis of the outstanding MR modalities (including NODDI, resting state and visual task fMRI, and arterial spin labelling), confirmation of the PET results using corrected parcellations, comparison of early PET uptake (as a measure of perfusion) in the patients and healthy volunteers, analysis of the neuropsychological testing and outcome measures collected. Additionally, there are likely to be new serum and CSF markers, developed for CAA or for other neurological diseases, which could be tested using the samples from BOCAA as part of larger, international collaborations. The results from the CSF analyses could have relevance for the mechanisms underlying CAA, and might direct future work that aims to answer two of the most fundamental outstanding questions in CAA: what is the mechanism behind vascular (as opposed to parenchymal) amyloid deposition, and why does vascular amyloid cause bleeding in a subset of patients? It will also be important to characterise the phenotypic heterogeneity of CAA, in particular the differences between patients presenting with cognitive versus haemorrhagic symptoms. Replication of the results from BOCAA in larger cohorts, as well as research investigating how the most promising of these biomarkers change with time, will be essential.

5.5 Final conclusions

SVDs have clinical relevance in diverse populations, and make important contributions to cognitive phenotype and prognosis. The recent development of novel technologies provides exciting new techniques for investigating SVDs, the outcome of which undoubtedly be fresh mechanistic insights into the manner in which they contribute to clinical disease. This progress will be key in our future ability to identify treatment targets in conditions for which there are currently no proven disease-modifying strategies; the hope is that this will lead to effective management and perhaps even curative treatment for patients diagnosed with these common age-related processes.

References

1. Libon DJ, Price CC, Davis Garrett K, Giovannetti T. From Binswanger's disease to leuokoaraiosis: what we have learned about subcortical vascular dementia. Clin Neuropsychol. 2004;18(1):83-100.
2. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. The Lancet Neurology. 2010;9(7):689-701.
3. Banerjee G, Wilson D, Jager HR, Werring DJ. Novel imaging techniques in cerebral small vessel diseases and vascular cognitive impairment. Biochimica et biophysica acta. 2016;1862(5):926-38.
4. World Alzheimer Report 2015: The Global Impact of Dementia, An analysis of prevalence, incid ence, cost and trends. 2015.
5. Feigin VL, Krishnamurthi RV, Parmar P, Norrving B, Mensah GA, Bennett DA, et al. Update on the Global Burden of Ischemic and Hemorrhagic Stroke in 1990-2013: The GBD 2013 Study. Neuroepidemiology. 2015;45(3):161-76.
6. Skrobot OA, Attems J, Esiri M, Hortobagyi T, Ironside JW, Kalaria RN, et al. Vascular cognitive impairment neuropathology guidelines (VCING): the contribution of cerebrovascular pathology to cognitive impairment. Brain : a journal of neurology. 2016;139(11):2957-69.
7. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. The Lancet Neurology. 2013;12(8):822-38.
8. Greenberg SM, Al-Shahi Salman R, Biessels GJ, van Buchem M, Cordonnier C, Lee JM, et al. Outcome markers for clinical trials in cerebral amyloid angiopathy. The Lancet Neurology. 2014;13(4):419-28.
9. Caplan LR. Lacunar infarction and small vessel disease: pathology and pathophysiology. Journal of stroke. 2015;17(1):2-6.
10. Wallin A, Ohrfelt A, Bjerke M. Characteristic clinical presentation and CSF biomarker pattern in cerebral small vessel disease. Journal of the neurological sciences. 2012;322(1-2):192-6.
11. Charidimou A, Werring DJ. Cerebral microbleeds and cognition in cerebrovascular disease: an update. Journal of the neurological sciences. 2012;322(1-2):50-5.
12. Wilson D, Charidimou A, Werring DJ. Advances in understanding spontaneous intracerebral hemorrhage: insights from neuroimaging. Expert Rev Neurother. 2014;14(6):661-78.
13. Charidimou A, Schmitt A, Wilson D, Yakushiji Y, Gregoire SM, Fox Z, et al. The Cerebral Haemorrhage Anatomical RaTing inStrument (CHARTS): Development and assessment of reliability. Journal of the neurological sciences. 2017;372:178-83.

14. Charidimou A, Gang Q, Werring DJ. Sporadic cerebral amyloid angiopathy revisited: recent insights into pathophysiology and clinical spectrum. Journal of neurology, neurosurgery, and psychiatry. 2012;83(2):124-37.
15. Wilson D, Hostettler I, Ambler G, Jager H, Werring D. Risk of intracerebral haemorrhage after acute convexity subarachnoid haemorrhage in suspected cerebral amyloid angiopathy: Hospital-based cohort study and meta-analysis. Int J Stroke. 2016;11(2):S25-S.
16. Yamada M. Cerebral amyloid angiopathy: emerging concepts. Journal of stroke. 2015;17(1):17-30.
17. Reijmer YD, van Veluw SJ, Greenberg SM. Ischemic brain injury in cerebral amyloid angiopathy. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2016;36(1):40-54.
18. Smith EE, Eichler F. Cerebral amyloid angiopathy and lobar intracerebral hemorrhage. Arch Neurol. 2006;63(1):148-51.
19. Lauer A, Greenberg SM, Gurol ME. Statins in Intracerebral Hemorrhage. Current atherosclerosis reports. 2015;17(8):46.
20. Poon MT, Fonville AF, Al-Shahi Salman R. Long-term prognosis after intracerebral haemorrhage: systematic review and meta-analysis. Journal of neurology, neurosurgery, and psychiatry. 2014;85(6):660-7.
21. Hemphill JC, 3rd, Greenberg SM, Anderson CS, Becker K, Bendok BR, Cushman M, et al. Guidelines for the Management of Spontaneous Intracerebral Hemorrhage: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke; a journal of cerebral circulation. 2015;46(7):2032-60.
22. Viswanathan A, Greenberg SM. Cerebral amyloid angiopathy in the elderly. Ann Neurol. 2011;70(6):871-80.
23. Biffi A, Kuramatsu JB, Leasure A, Kamel H, Kourkoulis C, Schwab K, et al. Oral Anticoagulation and Functional Outcome after Intracerebral Hemorrhage. Ann Neurol. 2017;82(5):755-65.
24. Bailey RD, Hart RG, Benavente O, Pearce LA. Recurrent brain hemorrhage is more frequent than ischemic stroke after intracranial hemorrhage. Neurology. 2001;56(6):773-7.
25. Hill MD, Silver FL, Austin PC, Tu JV. Rate of stroke recurrence in patients with primary intracerebral hemorrhage. Stroke; a journal of cerebral circulation. 2000;31(1):123-7.
26. Zia E, Engstrom G, Svensson PJ, Norrving B, Pessah-Rasmussen H. Three-year survival and stroke recurrence rates in patients with primary intracerebral hemorrhage. Stroke; a journal of cerebral circulation. 2009;40(11):3567-73.

27. Boyle PA, Yang J, Yu L, Leurgans SE, Capuano AW, Schneider JA, et al. Varied effects of age-related neuropathologies on the trajectory of late life cognitive decline. Brain : a journal of neurology. 2017;140(3):804-12.
28. Brott T, Thalinger K, Hertzberg V. Hypertension as a risk factor for spontaneous intracerebral hemorrhage. Stroke; a journal of cerebral circulation. 1986;17(6):1078-83.
29. Broderick J, Brott T, Tomsick T, Leach A. Lobar hemorrhage in the elderly. The undiminishing importance of hypertension. Stroke; a journal of cerebral circulation. 1993;24(1):49-51.
30. Kremer PH, Jolink WM, Kappelle LJ, Algra A, Klijn CJ, Smart, et al. Risk Factors for Lobar and Non-Lobar Intracerebral Hemorrhage in Patients with Vascular Disease. PloS one. 2015;10(11):e0142338.
31. Fisher CM. Pathological observations in hypertensive cerebral hemorrhage. J Neuropathol Exp Neurol. 1971;30(3):536-50.
32. Charidimou A, Linn J, Vernooij MW, Opherk C, Akoudad S, Baron JC, et al. Cortical superficial siderosis: detection and clinical significance in cerebral amyloid angiopathy and related conditions. Brain : a journal of neurology. 2015;138(Pt 8):2126-39.
33. Gregoire SM, Chaudhary UJ, Brown MM, Yousry TA, Kallis C, Jager HR, et al. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. Neurology. 2009;73(21):1759-66.
34. Charidimou A, Jager HR, Werring DJ. Cerebral microbleed detection and mapping: principles, methodological aspects and rationale in vascular dementia. Exp Gerontol. 2012;47(11):843-52.
35. Van der Flier WM, Cordonnier C. Microbleeds in vascular dementia: clinical aspects. Exp Gerontol. 2012;47(11):853-7.
36. Abbott NJ, Pizzo ME, Preston JE, Janigro D, Thorne RG. The role of brain barriers in fluid movement in the CNS: is there a 'glymphatic' system? Acta neuropathologica. 2018;135(3):387-407.
37. Weller RO, Hawkes CA, Kalaria RN, Werring DJ, Carare RO. White matter changes in dementia: role of impaired drainage of interstitial fluid. Brain Pathol. 2015;25(1):63-78.
38. Banerjee G, Kim HJ, Fox Z, Jager HR, Wilson D, Charidimou A, et al. MRI-visible perivascular space location is associated with Alzheimer's disease independently of amyloid burden. Brain : a journal of neurology. 2017;140(4):1107-16.
39. Bakker EN, Bacskai BJ, Arbel-Ornath M, Aldea R, Bedussi B, Morris AW, et al. Lymphatic Clearance of the Brain: Perivascular, Paravascular and Significance for Neurodegenerative Diseases. Cell Mol Neurobiol. 2016;36(2):181-94.

40. Charidimou A, Jaunmuktane Z, Baron J-C, Burnell M, Varlet P, Peeters A, et al. White matter perivascular spaces: an MRI marker in pathology-proven cerebral amyloid angiopathy? Neurology. 2013;In press.
41. Martinez-Ramirez S, Pontes-Neto OM, Dumas AP, Auriel E, Halpin A, Quimby M, et al. Topography of dilated perivascular spaces in subjects from a memory clinic cohort. Neurology. 2013;80(17):1551-6.
42. Charidimou A, Meegahage R, Fox Z, Peeters A, Vandermeeren Y, Laloux P, et al. Enlarged perivascular spaces as a marker of underlying arteriopathy in intracerebral haemorrhage: a multicentre MRI cohort study. Journal of neurology, neurosurgery, and psychiatry. 2013;84(6):624-9.
43. Imaizumi T, Inamura S, Kohama I, Yoshifuji K, Nomura T, Komatsu K. Nascent lobar microbleeds and stroke recurrences. J Stroke Cerebrovasc Dis. 2014;23(4):610-7.
44. Kim YJ, Kim HJ, Park JH, Kim S, Woo SY, Kwak KC, et al. Synergistic effects of longitudinal amyloid and vascular changes on lobar microbleeds. Neurology. 2016;87(15):1575-82.
45. Smith EE, Nandigam KR, Chen YW, Jeng J, Salat D, Halpin A, et al. MRI markers of small vessel disease in lobar and deep hemispheric intracerebral hemorrhage. Stroke; a journal of cerebral circulation. 2010;41(9):1933-8.
46. Tsai HH, Tsai LK, Chen YF, Tang SC, Lee BC, Yen RF, et al. Correlation of Cerebral Microbleed Distribution to Amyloid Burden in Patients with Primary Intracerebral Hemorrhage. Sci Rep. 2017;7:44715.
47. Potter GM, Doubal FN, Jackson CA, Chappell FM, Sudlow CL, Dennis MS, et al. Enlarged perivascular spaces and cerebral small vessel disease. International journal of stroke : official journal of the International Stroke Society. 2015;10(3):376-81.
48. Samarasekera N, Fonville A, Lerpiniere C, Farrall AJ, Wardlaw JM, White PM, et al. Influence of intracerebral hemorrhage location on incidence, characteristics, and outcome: population-based study. Stroke; a journal of cerebral circulation. 2015;46(2):361-8.
49. van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. The Lancet Neurology. 2010;9(2):167-76.
50. Boyle PA, Yu L, Nag S, Leurgans S, Wilson RS, Bennett DA, et al. Cerebral amyloid angiopathy and cognitive outcomes in community-based older persons. Neurology. 2015;85(22):1930-6.

51. Arvanitakis Z, Leurgans SE, Wang Z, Wilson RS, Bennett DA, Schneider JA. Cerebral amyloid angiopathy pathology and cognitive domains in older persons. Ann Neurol. 2011;69(2):320-7.
52. Chen YW, Gurol ME, Rosand J, Viswanathan A, Rakich SM, Groover TR, et al. Progression of white matter lesions and hemorrhages in cerebral amyloid angiopathy. Neurology. 2006;67(1):83-7.
53. Lee S, Viqar F, Zimmerman ME, Narkhede A, Tosto G, Benzinger TL, et al. White matter hyperintensities are a core feature of Alzheimer's disease: Evidence from the dominantly inherited Alzheimer network. Ann Neurol. 2016;79(6):929-39.
54. Thanprasertsuk S, Martinez-Ramirez S, Pontes-Neto OM, Ni J, Ayres A, Reed A, et al. Posterior white matter disease distribution as a predictor of amyloid angiopathy. Neurology. 2014;83(9):794-800.
55. Fotiadis P, van Rooden S, van der Grond J, Schultz A, Martinez-Ramirez S, Auriel E, et al. Cortical atrophy in patients with cerebral amyloid angiopathy: a case-control study. The Lancet Neurology. 2016;15(8):811-9.
56. Samuraki M, Matsunari I, Yoshita M, Shima K, Noguchi-Shinohara M, Hamaguchi T, et al. Cerebral Amyloid Angiopathy-Related Microbleeds Correlate with Glucose Metabolism and Brain Volume in Alzheimer's Disease. J Alzheimers Dis. 2015;48(2):517-28.
57. Linn J, Halpin A, Demaerel P, Ruhland J, Giese AD, Dichgans M, et al. Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy. Neurology. 2010;74(17):1346-50.
58. Rodrigues MA, Samarasekera N, Lerpiniere C, Humphreys C, McCarron MO, White PM, et al. The Edinburgh CT and genetic diagnostic criteria for lobar intracerebral haemorrhage associated with cerebral amyloid angiopathy: model development and diagnostic test accuracy study. The Lancet Neurology. 2018;17(3):232-40.
59. Knudsen KA, Rosand J, Karluk D, Greenberg SM. Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. Neurology. 2001;56(4):537-9.
60. Ni J, Auriel E, Jindal J, Ayres A, Schwab KM, Martinez-Ramirez S, et al. The characteristics of superficial siderosis and convexity subarachnoid hemorrhage and clinical relevance in suspected cerebral amyloid angiopathy. Cerebrovascular diseases. 2015;39(5-6):278-86.
61. Lummel N, Wollenweber FA, Demaerel P, Bochmann K, Malik R, Opherk C, et al. Clinical spectrum, underlying etiologies and radiological characteristics of cortical superficial siderosis. Journal of neurology. 2015;262(6):1455-62.
62. Calviere L, Cuvinciuc V, Raposo N, Faury A, Cognard C, Larrue V, et al. Acute Convexity Subarachnoid Hemorrhage Related to Cerebral Amyloid Angiopathy:

Clinicoradiological Features and Outcome. J Stroke Cerebrovasc Dis. 2016;25(5):1009-16.
63. Charidimou A, Boulouis G, Fotiadis P, Xiong L, Ayres AM, Schwab KM, et al. Acute convexity subarachnoid haemorrhage and cortical superficial siderosis in probable cerebral amyloid angiopathy without lobar haemorrhage. Journal of neurology, neurosurgery, and psychiatry. 2018;89(4):397-403.
64. Raposo N, Calviere L, Cazzola V, Planton M, Patsoura S, Wargny M, et al. Cortical superficial siderosis and acute convexity subarachnoid hemorrhage in cerebral amyloid angiopathy. Eur J Neurol. 2018;25(2):253-9.
65. Linn J, Wollenweber FA, Lummel N, Bochmann K, Pfefferkorn T, Gschwendtner A, et al. Superficial siderosis is a warning sign for future intracranial hemorrhage. Journal of neurology. 2013;260(1):176-81.
66. Charidimou A, Peeters AP, Jager R, Fox Z, Vandermeeren Y, Laloux P, et al. Cortical superficial siderosis and intracerebral hemorrhage risk in cerebral amyloid angiopathy. Neurology. 2013;81(19):1666-73.
67. Wilson D, Hostettler IC, Ambler G, Banerjee G, Jager HR, Werring DJ. Convexity subarachnoid haemorrhage has a high risk of intracerebral haemorrhage in suspected cerebral amyloid angiopathy. Journal of neurology. 2017;264(4):664-73.
68. Moulin S, Casolla B, Kuchcinski G, Boulouis G, Rossi C, Henon H, et al. Cortical superficial siderosis: A prospective observational cohort study. Neurology. 2018;91(2):e132-e8.
69. Charidimou A, Boulouis G, Roongpiboonsopit D, Auriel E, Pasi M, Haley K, et al. Cortical superficial siderosis multifocality in cerebral amyloid angiopathy: A prospective study. Neurology. 2017;89(21):2128-35.
70. Charidimou A, Boulouis G, Xiong L, Jessel MJ, Roongpiboonsopit D, Ayres A, et al. Cortical superficial siderosis and first-ever cerebral hemorrhage in cerebral amyloid angiopathy. Neurology. 2017;88(17):1607-14.
71. Roongpiboonsopit D, Charidimou A, William CM, Lauer A, Falcone GJ, Martinez-Ramirez S, et al. Cortical superficial siderosis predicts early recurrent lobar hemorrhage. Neurology. 2016;87(18):1863-70.
72. Na HK, Park JH, Kim JH, Kim HJ, Kim ST, Werring DJ, et al. Cortical superficial siderosis: a marker of vascular amyloid in patients with cognitive impairment. Neurology. 2015;84(8):849-55.
73. Pichler M, Vemuri P, Rabinstein AA, Aakre J, Flemming KD, Brown RD, Jr., et al. Prevalence and Natural History of Superficial Siderosis: A Population-Based Study. Stroke; a journal of cerebral circulation. 2017;48(12):3210-4.
74. Martinez-Ramirez S, Romero JR, Shoamanesh A, McKee AC, Van Etten E, Pontes-Neto O, et al. Diagnostic value of lobar microbleeds in individuals without

intracerebral hemorrhage. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2015;11(12):1480-8.
75. Wilson D, Werring DJ. Establishing the "meaning" of microbleeds: Clinical context or lobar microbleed burden? Alzheimer's & dementia : the journal of the Alzheimer's Association. 2016;12(1):85-6.
76. Carare RO, Hawkes CA, Jeffrey M, Kalaria RN, Weller RO. Review: cerebral amyloid angiopathy, prion angiopathy, CADASIL and the spectrum of protein elimination failure angiopathies (PEFA) in neurodegenerative disease with a focus on therapy. Neuropathol Appl Neurobiol. 2013;39(6):593-611.
77. Hawkes CA, Jayakody N, Johnston DA, Bechmann I, Carare RO. Failure of perivascular drainage of beta-amyloid in cerebral amyloid angiopathy. Brain Pathol. 2014;24(4):396-403.
78. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesions. The Lancet Neurology. 2012;11(3):272-82.
79. van Veluw SJ, Shih AY, Smith EE, Chen C, Schneider JA, Wardlaw JM, et al. Detection, risk factors, and functional consequences of cerebral microinfarcts. The Lancet Neurology. 2017;16(9):730-40.
80. Ferro DA, van Veluw SJ, Koek HL, Exalto LG, Biessels GJ, Utrecht Vascular Cognitive Impairment study g. Cortical Cerebral Microinfarcts on 3 Tesla MRI in Patients with Vascular Cognitive Impairment. J Alzheimers Dis. 2017;60(4):1443-50.
81. van Dalen JW, Scuric EE, van Veluw SJ, Caan MW, Nederveen AJ, Biessels GJ, et al. Cortical microinfarcts detected in vivo on 3 Tesla MRI: clinical and radiological correlates. Stroke; a journal of cerebral circulation. 2015;46(1):255-7.
82. van Veluw SJ, Hilal S, Kuijf HJ, Ikram MK, Xin X, Yeow TB, et al. Cortical microinfarcts on 3T MRI: Clinical correlates in memory-clinic patients. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2015;11(12):1500-9.
83. Hilal S, Sikking E, Shaik MA, Chan QL, van Veluw SJ, Vrooman H, et al. Cortical cerebral microinfarcts on 3T MRI: A novel marker of cerebrovascular disease. Neurology. 2016;87(15):1583-90.
84. van den Brink H, Zwiers A, Switzer AR, Charlton A, McCreary CR, Goodyear BG, et al. Cortical Microinfarcts on 3T Magnetic Resonance Imaging in Cerebral Amyloid Angiopathy. Stroke; a journal of cerebral circulation. 2018;49(8):1899-905.
85. Hecht M, Kramer LM, von Arnim CAF, Otto M, Thal DR. Capillary cerebral amyloid angiopathy in Alzheimer's disease: association with allocortical/hippocampal microinfarcts and cognitive decline. Acta neuropathologica. 2018;135(5):681-94.
86. Charidimou A, Martinez-Ramirez S, Reijmer YD, Oliveira-Filho J, Lauer A, Roongpiboonsopit D, et al. Total Magnetic Resonance Imaging Burden of Small Vessel

Disease in Cerebral Amyloid Angiopathy: An Imaging-Pathologic Study of Concept Validation. JAMA neurology. 2016;73(8):994-1001.
87. Banerjee G, Carare R, Cordonnier C, Greenberg SM, Schneider JA, Smith EE, et al. The increasing impact of cerebral amyloid angiopathy: essential new insights for clinical practice. Journal of neurology, neurosurgery, and psychiatry. 2017;88(11):982-94.
88. Charidimou A, Jager RH, Peeters A, Vandermeeren Y, Laloux P, Baron JC, et al. White matter perivascular spaces are related to cortical superficial siderosis in cerebral amyloid angiopathy. Stroke; a journal of cerebral circulation. 2014;45(10):2930-5.
89. Charidimou A, Jaunmuktane Z, Baron JC, Burnell M, Varlet P, Peeters A, et al. White matter perivascular spaces: an MRI marker in pathology-proven cerebral amyloid angiopathy? Neurology. 2014;82(1):57-62.
90. Koo HW, Jo KI, Yeon JY, Kim JS, Hong SC. Clinical features of high-degree centrum semiovale-perivascular spaces in cerebral amyloid angiopathy. Journal of the neurological sciences. 2016;367:89-94.
91. Shams S, Martola J, Charidimou A, Larvie M, Granberg T, Shams M, et al. Topography and Determinants of Magnetic Resonance Imaging (MRI)-Visible Perivascular Spaces in a Large Memory Clinic Cohort. Journal of the American Heart Association. 2017;6(9).
92. Charidimou A, Boulouis G, Pasi M, Auriel E, van Etten ES, Haley K, et al. MRI-visible perivascular spaces in cerebral amyloid angiopathy and hypertensive arteriopathy. Neurology. 2017;88(12):1157-64.
93. Martinez-Ramirez S, van Rooden S, Charidimou A, van Opstal AM, Wermer M, Gurol ME, et al. Perivascular Spaces Volume in Sporadic and Hereditary (Dutch-Type) Cerebral Amyloid Angiopathy. Stroke; a journal of cerebral circulation. 2018;49(8):1913-9.
94. Charidimou A, Hong YT, Jager HR, Fox Z, Aigbirhio FI, Fryer TD, et al. White matter perivascular spaces on magnetic resonance imaging: marker of cerebrovascular amyloid burden? Stroke; a journal of cerebral circulation. 2015;46(6):1707-9.
95. Kim J, Na HK, Shin JH, Kim HJ, Seo SW, Seong JK, et al. Atrophy patterns in cerebral amyloid angiopathy with and without cortical superficial siderosis. Neurology. 2018;90(20):e1751-e8.
96. Auriel E, Edlow BL, Reijmer YD, Fotiadis P, Ramirez-Martinez S, Ni J, et al. Microinfarct disruption of white matter structure: a longitudinal diffusion tensor analysis. Neurology. 2014;83(2):182-8.
97. van Veluw SJ, Charidimou A, van der Kouwe AJ, Lauer A, Reijmer YD, Costantino I, et al. Microbleed and microinfarct detection in amyloid angiopathy: a high-

resolution MRI-histopathology study. Brain : a journal of neurology. 2016;139(Pt 12):3151-62.
98. Peca S, McCreary CR, Donaldson E, Kumarpillai G, Shobha N, Sanchez K, et al. Neurovascular decoupling is associated with severity of cerebral amyloid angiopathy. Neurology. 2013;81(19):1659-65.
99. Dumas A, Dierksen GA, Gurol ME, Halpin A, Martinez-Ramirez S, Schwab K, et al. Functional magnetic resonance imaging detection of vascular reactivity in cerebral amyloid angiopathy. Ann Neurol. 2012;72(1):76-81.
100. Switzer AR, McCreary C, Batool S, Stafford RB, Frayne R, Goodyear BG, et al. Longitudinal decrease in blood oxygenation level dependent response in cerebral amyloid angiopathy. NeuroImage Clinical. 2016;11:461-7.
101. Williams RJ, Goodyear BG, Peca S, McCreary CR, Frayne R, Smith EE, et al. Identification of neurovascular changes associated with cerebral amyloid angiopathy from subject-specific hemodynamic response functions. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2017;37(10):3433-45.
102. Reijmer YD, Fotiadis P, Martinez-Ramirez S, Salat DH, Schultz A, Shoamanesh A, et al. Structural network alterations and neurological dysfunction in cerebral amyloid angiopathy. Brain : a journal of neurology. 2015;138(Pt 1):179-88.
103. Valenti R, Reijmer YD, Charidimou A, Boulouis G, Martinez SR, Xiong L, et al. Total small vessel disease burden and brain network efficiency in cerebral amyloid angiopathy. Journal of the neurological sciences. 2017;382:10-2.
104. Reijmer YD, Fotiadis P, Riley GA, Xiong L, Charidimou A, Boulouis G, et al. Progression of Brain Network Alterations in Cerebral Amyloid Angiopathy. Stroke; a journal of cerebral circulation. 2016;47(10):2470-5.
105. Reijmer YD, Fotiadis P, Charidimou A, van Veluw SJ, Xiong L, Riley GA, et al. Relationship between white matter connectivity loss and cortical thinning in cerebral amyloid angiopathy. Hum Brain Mapp. 2017.
106. Charidimou A, Farid K, Baron JC. Amyloid-PET in sporadic cerebral amyloid angiopathy: A diagnostic accuracy meta-analysis. Neurology. 2017;89(14):1490-8.
107. Gurol ME, Dierksen G, Betensky R, Gidicsin C, Halpin A, Becker A, et al. Predicting sites of new hemorrhage with amyloid imaging in cerebral amyloid angiopathy. Neurology. 2012;79(4):320-6.
108. Baron JC, Farid K, Dolan E, Turc G, Marrapu ST, O'Brien E, et al. Diagnostic utility of amyloid PET in cerebral amyloid angiopathy-related symptomatic intracerebral hemorrhage. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2014;34(5):753-8.

109. Farid K, Hong YT, Aigbirhio FI, Fryer TD, Menon DK, Warburton EA, et al. Early-Phase 11C-PiB PET in Amyloid Angiopathy-Related Symptomatic Cerebral Hemorrhage: Potential Diagnostic Value? PloS one. 2015;10(10):e0139926.
110. Charidimou A, Farid K, Tsai HH, Tsai LK, Yen RF, Baron JC. Amyloid-PET burden and regional distribution in cerebral amyloid angiopathy: a systematic review and meta-analysis of biomarker performance. Journal of neurology, neurosurgery, and psychiatry. 2018;89(4):410-7.
111. Gurol ME, Becker JA, Fotiadis P, Riley G, Schwab K, Johnson KA, et al. Florbetapir-PET to diagnose cerebral amyloid angiopathy: A prospective study. Neurology. 2016;87(19):2043-9.
112. Raposo N, Planton M, Peran P, Payoux P, Bonneville F, Lyoubi A, et al. Florbetapir imaging in cerebral amyloid angiopathy-related hemorrhages. Neurology. 2017;89(7):697-704.
113. Simonsen AH, Herukka SK, Andreasen N, Baldeiras I, Bjerke M, Blennow K, et al. Recommendations for CSF AD biomarkers in the diagnostic evaluation of dementia. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2017;13(3):274-84.
114. Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: current status and prospects for the future. Journal of internal medicine. 2018;284(6):643-63.
115. Sperling R, Salloway S, Brooks DJ, Tampieri D, Barakos J, Fox NC, et al. Amyloid-related imaging abnormalities in patients with Alzheimer's disease treated with bapineuzumab: a retrospective analysis. The Lancet Neurology. 2012;11(3):241-9.
116. Sevigny J, Chiao P, Bussiere T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. Nature. 2016;537(7618):50-6.
117. DiFrancesco JC, Longoni M, Piazza F. Anti-Abeta Autoantibodies in Amyloid Related Imaging Abnormalities (ARIA): Candidate Biomarker for Immunotherapy in Alzheimer's Disease and Cerebral Amyloid Angiopathy. Front Neurol. 2015;6:207.
118. Corovic A, Kelly S, Markus HS. Cerebral amyloid angiopathy associated with inflammation: A systematic review of clinical and imaging features and outcome. International journal of stroke : official journal of the International Stroke Society. 2018;13(3):257-67.
119. Piazza F, Greenberg SM, Savoiardo M, Gardinetti M, Chiapparini L, Raicher I, et al. Anti-amyloid beta autoantibodies in cerebral amyloid angiopathy-related inflammation: implications for amyloid-modifying therapies. Ann Neurol. 2013;73(4):449-58.
120. Banerjee G, Alvares D, Bowen J, Adams ME, Werring DJ. Minimally symptomatic cerebral amyloid angiopathy-related inflammation: three descriptive case reports. Journal of neurology, neurosurgery, and psychiatry. 2019;90(1):113-5.

121. van Veluw SJ, Kuijf HJ, Charidimou A, Viswanathan A, Biessels GJ, Rozemuller AJ, et al. Reduced vascular amyloid burden at microhemorrhage sites in cerebral amyloid angiopathy. Acta neuropathologica. 2017;133(3):409-15.
122. van Etten ES, Verbeek MM, van der Grond J, Zielman R, van Rooden S, van Zwet EW, et al. beta-Amyloid in CSF: Biomarker for preclinical cerebral amyloid angiopathy. Neurology. 2017;88(2):169-76.
123. Moulin S, Labreuche J, Bombois S, Rossi C, Boulouis G, Henon H, et al. Dementia risk after spontaneous intracerebral haemorrhage: a prospective cohort study. The Lancet Neurology. 2016;15(8):820-9.
124. Biffi A, Bailey D, Anderson CD, Ayres AM, Gurol EM, Greenberg SM, et al. Risk Factors Associated With Early vs Delayed Dementia After Intracerebral Hemorrhage. JAMA neurology. 2016;73(8):969-76.
125. Case NF, Charlton A, Zwiers A, Batool S, McCreary CR, Hogan DB, et al. Cerebral Amyloid Angiopathy Is Associated With Executive Dysfunction and Mild Cognitive Impairment. Stroke; a journal of cerebral circulation. 2016;47(8):2010-6.
126. Kapasi A, DeCarli C, Schneider JA. Impact of multiple pathologies on the threshold for clinically overt dementia. Acta neuropathologica. 2017;134(2):171-86.
127. Smith EE. Cerebral amyloid angiopathy as a cause of neurodegeneration. J Neurochem. 2018;144(5):651-8.
128. Xiong L, Davidsdottir S, Reijmer YD, Shoamanesh A, Roongpiboonsopit D, Thanprasertsuk S, et al. Cognitive Profile and its Association with Neuroimaging Markers of Non-Demented Cerebral Amyloid Angiopathy Patients in a Stroke Unit. J Alzheimers Dis. 2016;52(1):171-8.
129. Ganz AB, Beker N, Hulsman M, Sikkes S, Netherlands Brain B, Scheltens P, et al. Neuropathology and cognitive performance in self-reported cognitively healthy centenarians. Acta neuropathologica communications. 2018;6(1):64.
130. Valenti R, Charidimou A, Xiong L, Boulouis G, Fotiadis P, Ayres A, et al. Visuospatial Functioning in Cerebral Amyloid Angiopathy: A Pilot Study. J Alzheimers Dis. 2017;56(4):1223-7.
131. Planton M, Raposo N, Albucher JF, Pariente J. Cerebral amyloid angiopathy-related cognitive impairment: The search for a specific neuropsychological pattern. Rev Neurol (Paris). 2017;173(9):562-5.
132. Banerjee G, Summers M, Chan E, Wilson D, Charidimou A, Cipolotti L, et al. Domain-specific characterisation of early cognitive impairment following spontaneous intracerebral haemorrhage. Journal of the neurological sciences. 2018;391:25-30.
133. Xiong L, Reijmer YD, Charidimou A, Cordonnier C, Viswanathan A. Intracerebral hemorrhage and cognitive impairment. Biochimica et biophysica acta. 2016;1862(5):939-44.

134. Planton M, Saint-Aubert L, Raposo N, Branchu L, Lyoubi A, Bonneville F, et al. High prevalence of cognitive impairment after intracerebral hemorrhage. PloS one. 2017;12(6):e0178886.
135. Xiong L, Boulouis G, Charidimou A, Roongpiboonsopit D, Jessel MJ, Pasi M, et al. Dementia incidence and predictors in cerebral amyloid angiopathy patients without intracerebral hemorrhage. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2018;38(2):241-9.
136. Gahr M, Nowak DA, Connemann BJ, Schonfeldt-Lecuona C. Cerebral Amyloidal Angiopathy--a disease with implications for neurology and psychiatry. Brain Res. 2013;1519:19-30.
137. Gahr M, Connemann BJ, Schonfeldt-Lecuona C. [Behavioural problems and personality change related to cerebral amyloid angiopathy]. Psychiatr Prax. 2012;39(8):410-3.
138. Gleason A, Hayhow B, Emmanuel J, Gaillard F. Cerebral amyloid angiopathy presenting with neuropsychiatric symptoms. Aust N Z J Psychiatry. 2014;48(8):779-80.
139. Vik-Mo AO, Bencze J, Ballard C, Hortobagyi T, Aarsland D. Advanced cerebral amyloid angiopathy and small vessel disease are associated with psychosis in Alzheimer's disease. Journal of neurology, neurosurgery, and psychiatry. 2018.
140. Alexopoulos GS, Meyers BS, Young RC, Campbell S, Silbersweig D, Charlson M. 'Vascular depression' hypothesis. Arch Gen Psychiatry. 1997;54(10):915-22.
141. Brookes RL, Herbert V, Lawrence AJ, Morris RG, Markus HS. Depression in small-vessel disease relates to white matter ultrastructural damage, not disability. Neurology. 2014;83(16):1417-23.
142. van Sloten TT, Sigurdsson S, van Buchem MA, Phillips CL, Jonsson PV, Ding J, et al. Cerebral Small Vessel Disease and Association With Higher Incidence of Depressive Symptoms in a General Elderly Population: The AGES-Reykjavik Study. Am J Psychiatry. 2015;172(6):570-8.
143. Zhang X, Tang Y, Xie Y, Ding C, Xiao J, Jiang X, et al. Total magnetic resonance imaging burden of cerebral small-vessel disease is associated with post-stroke depression in patients with acute lacunar stroke. Eur J Neurol. 2017;24(2):374-80.
144. Direk N, Perez HS, Akoudad S, Verhaaren BF, Niessen WJ, Hofman A, et al. Markers of cerebral small vessel disease and severity of depression in the general population. Psychiatry research Neuroimaging. 2016;253:1-6.
145. Grool AM, van der Graaf Y, Mali WP, Witkamp TD, Vincken KL, Geerlings MI, et al. Location and progression of cerebral small-vessel disease and atrophy, and

depressive symptom profiles: the Second Manifestations of ARTerial disease (SMART)-Medea study. Psychol Med. 2012;42(2):359-70.
146. van Agtmaal MJM, Houben A, Pouwer F, Stehouwer CDA, Schram MT. Association of Microvascular Dysfunction With Late-Life Depression: A Systematic Review and Meta-analysis. JAMA psychiatry. 2017;74(7):729-39.
147. Rutten-Jacobs LCA, Tozer DJ, Duering M, Malik R, Dichgans M, Markus HS, et al. Genetic Study of White Matter Integrity in UK Biobank (N=8448) and the Overlap With Stroke, Depression, and Dementia. Stroke; a journal of cerebral circulation. 2018;49(6):1340-7.
148. Leeuwis AE, Prins ND, Hooghiemstra AM, Benedictus MR, Scheltens P, Barkhof F, et al. Microbleeds are associated with depressive symptoms in Alzheimer's disease. Alzheimer's & dementia (Amsterdam, Netherlands). 2018;10:112-20.
149. Wang R, Liu K, Ye X, Yan S. Association Between Cerebral Microbleeds and Depression in the General Elderly Population: A Meta-Analysis. Frontiers in psychiatry. 2018;9:94.
150. Verbeek MM, Kremer BP, Rikkert MO, Van Domburg PH, Skehan ME, Greenberg SM. Cerebrospinal fluid amyloid beta(40) is decreased in cerebral amyloid angiopathy. Ann Neurol. 2009;66(2):245-9.
151. Charidimou A, Friedrich JO, Greenberg SM, Viswanathan A. Core cerebrospinal fluid biomarker profile in cerebral amyloid angiopathy: A meta-analysis. Neurology. 2018;90(9):e754-e62.
152. Renard D, Castelnovo G, Wacongne A, Le Floch A, Thouvenot E, Mas J, et al. Interest of CSF biomarker analysis in possible cerebral amyloid angiopathy cases defined by the modified Boston criteria. Journal of neurology. 2012;259(11):2429-33.
153. Renard D, Gabelle A, Hirtz C, Demattei C, Thouvenot E, Lehmann S. Cerebrospinal Fluid Alzheimer's Disease Biomarkers in Isolated Supratentorial Cortical Superficial Siderosis. J Alzheimers Dis. 2016;54(4):1291-5.
154. Noguchi-Shinohara M, Komatsu J, Samuraki M, Matsunari I, Ikeda T, Sakai K, et al. Cerebral Amyloid Angiopathy-Related Microbleeds and Cerebrospinal Fluid Biomarkers in Alzheimer's Disease. J Alzheimers Dis. 2017;55(3):905-13.
155. Goos JD, Kester MI, Barkhof F, Klein M, Blankenstein MA, Scheltens P, et al. Patients with Alzheimer disease with multiple microbleeds: relation with cerebrospinal fluid biomarkers and cognition. Stroke; a journal of cerebral circulation. 2009;40(11):3455-60.
156. Shams S, Granberg T, Martola J, Charidimou A, Li X, Shams M, et al. Cerebral microbleeds topography and cerebrospinal fluid biomarkers in cognitive impairment. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2017;37(3):1006-13.

157. Goos JD, Teunissen CE, Veerhuis R, Verwey NA, Barkhof F, Blankenstein MA, et al. Microbleeds relate to altered amyloid-beta metabolism in Alzheimer's disease. Neurobiology of aging. 2012;33(5):1011 e1-9.
158. Kester MI, Goos JD, Teunissen CE, Benedictus MR, Bouwman FH, Wattjes MP, et al. Associations between cerebral small-vessel disease and Alzheimer disease pathology as measured by cerebrospinal fluid biomarkers. JAMA neurology. 2014;71(7):855-62.
159. Shams S, Martola J, Charidimou A, Cavallin L, Granberg T, Shams M, et al. Cortical superficial siderosis: Prevalence and biomarker profile in a memory clinic population. Neurology. 2016;87(11):1110-7.
160. Bornebroek M, De Jonghe C, Haan J, Kumar-Singh S, Younkin S, Roos R, et al. Hereditary cerebral hemorrhage with amyloidosis Dutch type (AbetaPP 693): decreased plasma amyloid-beta 42 concentration. Neurobiol Dis. 2003;14(3):619-23.
161. Hernandez-Guillamon M, Delgado P, Penalba A, Rodriguez-Luna D, Molina CA, Rovira A, et al. Plasma beta-amyloid levels in cerebral amyloid angiopathy-associated hemorrhagic stroke. Neurodegener Dis. 2012;10(1-4):320-3.
162. Banerjee G, Jang H, Kim HJ, Kim ST, Kim JS, Lee JH, et al. Total MRI Small Vessel Disease Burden Correlates with Cognitive Performance, Cortical Atrophy, and Network Measures in a Memory Clinic Population. J Alzheimers Dis. 2018;63(4):1485-97.
163. Economic impact - World Alzheimer Report 2010 2010 [Available from: http://www.alz.co.uk/research/world-report.
164. Wu YT, Fratiglioni L, Matthews FE, Lobo A, Breteler MM, Skoog I, et al. Dementia in western Europe: epidemiological evidence and implications for policy making. The Lancet Neurology. 2016;15(1):116-24.
165. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet neurology. 2014;13(8):788-94.
166. Makin SD, Turpin S, Dennis MS, Wardlaw JM. Cognitive impairment after lacunar stroke: systematic review and meta-analysis of incidence, prevalence and comparison with other stroke subtypes. Journal of neurology, neurosurgery, and psychiatry. 2013;84(8):893-900.
167. Arboix A. Lacunar infarct and cognitive decline. Expert Rev Neurother. 2011;11(9):1251-4.
168. Edwards JD, Jacova C, Sepehry AA, Pratt B, Benavente OR. A quantitative systematic review of domain-specific cognitive impairment in lacunar stroke. Neurology. 2013;80(3):315-22.

169. Prins ND, Scheltens P. White matter hyperintensities, cognitive impairment and dementia: an update. Nat Rev Neurol. 2015;11(3):157-65.
170. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. Bmj. 2010;341:c3666.
171. Schmidt R, Petrovic K, Ropele S, Enzinger C, Fazekas F. Progression of leukoaraiosis and cognition. Stroke; a journal of cerebral circulation. 2007;38(9):2619-25.
172. Kloppenborg RP, Nederkoorn PJ, Grool AM, Vincken KL, Mali WP, Vermeulen M, et al. Cerebral small-vessel disease and progression of brain atrophy: the SMART-MR study. Neurology. 2012;79(20):2029-36.
173. Jokinen H, Lipsanen J, Schmidt R, Fazekas F, Gouw AA, van der Flier WM, et al. Brain atrophy accelerates cognitive decline in cerebral small vessel disease: the LADIS study. Neurology. 2012;78(22):1785-92.
174. The LSG, Poggesi A, Pantoni L, Inzitari D, Fazekas F, Ferro J, et al. 2001-2011: A Decade of the LADIS (Leukoaraiosis And DISability) Study: What Have We Learned about White Matter Changes and Small-Vessel Disease? Cerebrovascular diseases. 2011;32(6):577-88.
175. Nitkunan A, Lanfranconi S, Charlton RA, Barrick TR, Markus HS. Brain atrophy and cerebral small vessel disease: a prospective follow-up study. Stroke; a journal of cerebral circulation. 2011;42(1):133-8.
176. Appelman AP, Exalto LG, van der Graaf Y, Biessels GJ, Mali WP, Geerlings MI. White matter lesions and brain atrophy: more than shared risk factors? A systematic review. Cerebrovascular diseases. 2009;28(3):227-42.
177. Appelman AP, Vincken KL, van der Graaf Y, Vlek AL, Witkamp TD, Mali WP, et al. White matter lesions and lacunar infarcts are independently and differently associated with brain atrophy: the SMART-MR study. Cerebrovascular diseases. 2010;29(1):28-35.
178. Aribisala BS, Valdes Hernandez MC, Royle NA, Morris Z, Munoz Maniega S, Bastin ME, et al. Brain atrophy associations with white matter lesions in the ageing brain: the Lothian Birth Cohort 1936. Eur Radiol. 2013;23(4):1084-92.
179. Staals J, Makin SD, Doubal FN, Dennis MS, Wardlaw JM. Stroke subtype, vascular risk factors, and total MRI brain small-vessel disease burden. Neurology. 2014;83(14):1228-34.
180. Huijts M, Duits A, van Oostenbrugge RJ, Kroon AA, de Leeuw PW, Staals J. Accumulation of MRI Markers of Cerebral Small Vessel Disease is Associated with Decreased Cognitive Function. A Study in First-Ever Lacunar Stroke and Hypertensive Patients. Front Aging Neurosci. 2013;5:72.

181. Staals J, Booth T, Morris Z, Bastin ME, Gow AJ, Corley J, et al. Total MRI load of cerebral small vessel disease and cognitive ability in older people. Neurobiology of aging. 2015;36(10):2806-11.
182. Uiterwijk R, van Oostenbrugge RJ, Huijts M, De Leeuw PW, Kroon AA, Staals J. Total Cerebral Small Vessel Disease MRI Score Is Associated with Cognitive Decline in Executive Function in Patients with Hypertension. Front Aging Neurosci. 2016;8:301.
183. Del Brutto VJ, Ortiz JG, Del Brutto OH, Mera RM, Zambrano M, Biller J. Total cerebral small vessel disease score and cognitive performance in community-dwelling older adults. Results from the Atahualpa Project. Int J Geriatr Psychiatry. 2018;33(2):325-31.
184. Lau KK, Li L, Schulz U, Simoni M, Chan KH, Ho SL, et al. Total small vessel disease score and risk of recurrent stroke: Validation in 2 large cohorts. Neurology. 2017;88(24):2260-7.
185. Hatate J, Miwa K, Matsumoto M, Sasaki T, Yagita Y, Sakaguchi M, et al. Association between cerebral small vessel diseases and mild parkinsonian signs in the elderly with vascular risk factors. Parkinsonism Relat Disord. 2016;26:29-34.
186. Loos CM, McHutchison C, Cvoro V, Makin SD, Staals J, Chappell F, et al. The relation between total cerebral small vessel disease burden and gait impairment in patients with minor stroke. International journal of stroke : official journal of the International Stroke Society. 2018;13(5):518-24.
187. Song TJ, Kim J, Song D, Yoo J, Lee HS, Kim YJ, et al. Total Cerebral Small-Vessel Disease Score is Associated with Mortality during Follow-Up after Acute Ischemic Stroke. J Clin Neurol. 2017;13(2):187-95.
188. Yakushiji Y, Charidimou A, Noguchi T, Nishihara M, Eriguchi M, Nanri Y, et al. Total Small Vessel Disease Score in Neurologically Healthy Japanese Adults in the Kashima Scan Study. Intern Med. 2018;57(2):189-96.
189. Boulouis G, Charidimou A, Jessel MJ, Xiong L, Roongpiboonsopit D, Fotiadis P, et al. Small vessel disease burden in cerebral amyloid angiopathy without symptomatic hemorrhage. Neurology. 2017;88(9):878-84.
190. Boulouis G, Charidimou A, Pasi M, Roongpiboonsopit D, Xiong L, Auriel E, et al. Hemorrhage recurrence risk factors in cerebral amyloid angiopathy: Comparative analysis of the overall small vessel disease severity score versus individual neuroimaging markers. Journal of the neurological sciences. 2017;380:64-7.
191. Banerjee G, Wilson D, Ambler G, Osei-Bonsu Appiah K, Shakeshaft C, Lunawat S, et al. Cognitive Impairment Before Intracerebral Hemorrhage Is Associated With Cerebral Amyloid Angiopathy. Stroke; a journal of cerebral circulation. 2018;49(1):40-5.

192. Doubal FN, MacLullich AM, Ferguson KJ, Dennis MS, Wardlaw JM. Enlarged perivascular spaces on MRI are a feature of cerebral small vessel disease. Stroke; a journal of cerebral circulation. 2010;41(3):450-4.
193. Maclullich AM, Wardlaw JM, Ferguson KJ, Starr JM, Seckl JR, Deary IJ. Enlarged perivascular spaces are associated with cognitive function in healthy elderly men. Journal of neurology, neurosurgery, and psychiatry. 2004;75(11):1519-23.
194. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. AJR Am J Roentgenol. 1987;149(2):351-6.
195. Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, et al. Pathologic correlates of incidental MRI white matter signal hyperintensities. Neurology. 1993;43(9):1683-9.
196. Harper L, Barkhof F, Fox NC, Schott JM. Using visual rating to diagnose dementia: a critical evaluation of MRI atrophy scales. Journal of neurology, neurosurgery, and psychiatry. 2015;86(11):1225-33.
197. Kebets V, Gregoire SM, Charidimou A, Barnes J, Rantell K, Brown MM, et al. Prevalence and cognitive impact of medial temporal atrophy in a hospital stroke service: retrospective cohort study. International journal of stroke : official journal of the International Stroke Society. 2015;10(6):861-7.
198. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. Jama. 1997;277(10):813-7.
199. Lee MJ, Seo SW, Na DL, Kim C, Park JH, Kim GH, et al. Synergistic effects of ischemia and beta-amyloid burden on cognitive decline in patients with subcortical vascular mild cognitive impairment. JAMA psychiatry. 2014;71(4):412-22.
200. Liu W, Wong A, Law AC, Mok VC. Cerebrovascular disease, amyloid plaques, and dementia. Stroke; a journal of cerebral circulation. 2015;46(5):1402-7.
201. Kalaria RN. Neuropathological diagnosis of vascular cognitive impairment and vascular dementia with implications for Alzheimer's disease. Acta neuropathologica. 2016;131(5):659-85.
202. Kim YJ, Kwon HK, Lee JM, Kim YJ, Kim HJ, Jung NY, et al. White matter microstructural changes in pure Alzheimer's disease and subcortical vascular dementia. Eur J Neurol. 2015;22(4):709-16.
203. Graham NL, Emery T, Hodges JR. Distinctive cognitive profiles in Alzheimer's disease and subcortical vascular dementia. Journal of neurology, neurosurgery, and psychiatry. 2004;75(1):61-71.

204. Ahmed RM, Paterson RW, Warren JD, Zetterberg H, O'Brien JT, Fox NC, et al. Biomarkers in dementia: clinical utility and new directions. Journal of neurology, neurosurgery, and psychiatry. 2014;85(12):1426-34.
205. Andrieu S, Coley N, Lovestone S, Aisen PS, Vellas B. Prevention of sporadic Alzheimer's disease: lessons learned from clinical trials and future directions. The Lancet Neurology. 2015;14(9):926-44.
206. Roher AE, Kuo YM, Esh C, Knebel C, Weiss N, Kalback W, et al. Cortical and leptomeningeal cerebrovascular amyloid and white matter pathology in Alzheimer's disease. Mol Med. 2003;9(3-4):112-22.
207. Ramirez J, Berezuk C, McNeely AA, Scott CJ, Gao F, Black SE. Visible Virchow-Robin spaces on magnetic resonance imaging of Alzheimer's disease patients and normal elderly from the Sunnybrook Dementia Study. J Alzheimers Dis. 2015;43(2):415-24.
208. Yakushiji Y, Charidimou A, Hara M, Noguchi T, Nishihara M, Eriguchi M, et al. Topography and associations of perivascular spaces in healthy adults: the Kashima scan study. Neurology. 2014;83(23):2116-23.
209. van Veluw SJ, Biessels GJ, Bouvy WH, Spliet WG, Zwanenburg JJ, Luijten PR, et al. Cerebral amyloid angiopathy severity is linked to dilation of juxtacortical perivascular spaces. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2016;36(3):576-80.
210. Kim HJ, Im K, Kwon H, Lee JM, Kim C, Kim YJ, et al. Clinical effect of white matter network disruption related to amyloid and small vessel disease. Neurology. 2015;85(1):63-70.
211. Seo SW, Cho SS, Park A, Chin J, Na DL. Subcortical vascular versus amnestic mild cognitive impairment: comparison of cerebral glucose metabolism. J Neuroimaging. 2009;19(3):213-9.
212. Erkinjuntti T, Inzitari D, Pantoni L, Wallin A, Scheltens P, Rockwood K, et al. Research criteria for subcortical vascular dementia in clinical trials. Advances in Dementia Research: Springer; 2000. p. 23-30.
213. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984;34(7):939-.
214. Collins DL, Neelin P, Peters TM, Evans AC. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr. 1994;18(2):192-205.

215. Johnson KA, Gregas M, Becker JA, Kinnecom C, Salat DH, Moran EK, et al. Imaging of amyloid burden and distribution in cerebral amyloid angiopathy. Ann Neurol. 2007;62(3):229-34.
216. Jeynes B, Provias J. The possible role of capillary cerebral amyloid angiopathy in Alzheimer lesion development: a regional comparison. Acta neuropathologica. 2006;112(4):417-27.
217. Kurian M, Burkhardt K, Assal F, Kovari E, Horvath J. Amyloid plaques and intraneuronal tau inclusions in A-beta-related angiitis (ABRA). Neuropathol Appl Neurobiol. 2012;38(4):391-4.
218. Williams S, Chalmers K, Wilcock GK, Love S. Relationship of neurofibrillary pathology to cerebral amyloid angiopathy in Alzheimer's disease. Neuropathol Appl Neurobiol. 2005;31(4):414-21.
219. Weller RO, Preston SD, Subash M, Carare RO. Cerebral amyloid angiopathy in the aetiology and immunotherapy of Alzheimer disease. Alzheimers Res Ther. 2009;1(2):6.
220. Love S, Miners JS. Cerebrovascular disease in ageing and Alzheimer's disease. Acta neuropathologica. 2016;131(5):645-58.
221. Iliff JJ, Chen MJ, Plog BA, Zeppenfeld DM, Soltero M, Yang L, et al. Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2014;34(49):16180-93.
222. Kress BT, Iliff JJ, Xia M, Wang M, Wei HS, Zeppenfeld D, et al. Impairment of paravascular clearance pathways in the aging brain. Ann Neurol. 2014;76(6):845-61.
223. Iqbal K, Liu F, Gong CX. Tau and neurodegenerative disease: the story so far. Nat Rev Neurol. 2016;12(1):15-27.
224. Thrane AS, Rangroo Thrane V, Nedergaard M. Drowning stars: reassessing the role of astrocytes in brain edema. Trends Neurosci. 2014;37(11):620-8.
225. Arima K, Izumiyama Y, Nakamura M, Nakayama H, Kimura M, Ando S, et al. Argyrophilic tau-positive twisted and non-twisted tubules in astrocytic processes in brains of Alzheimer-type dementia: an electron microscopical study. Acta neuropathologica. 1998;95(1):28-39.
226. Pendlebury ST, Rothwell PM. Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. The Lancet Neurology. 2009;8(11):1006-18.
227. Charidimou A, Wilson D, Shakeshaft C, Ambler G, White M, Cohen H, et al. The Clinical Relevance of Microbleeds in Stroke study (CROMIS-2): rationale, design, and methods. International journal of stroke : official journal of the International Stroke Society. 2015;10 Suppl A100:155-61.

228. McGovern A, Pendlebury ST, Mishra NK, Fan Y, Quinn TJ. Test Accuracy of Informant-Based Cognitive Screening Tests for Diagnosis of Dementia and Multidomain Cognitive Impairment in Stroke. Stroke; a journal of cerebral circulation. 2016;47(2):329-35.
229. Harrison JK, Fearon P, Noel-Storr AH, McShane R, Stott DJ, Quinn TJ. Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) for the diagnosis of dementia within a secondary care setting. The Cochrane database of systematic reviews. 2015;3(3):CD010772.
230. Jorm AF. A short form of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): development and cross-validation. Psychol Med. 1994;24(1):145-53.
231. Volbers B, Staykov D, Wagner I, Dorfler A, Saake M, Schwab S, et al. Semi-automatic volumetric assessment of perihemorrhagic edema with computed tomography. Eur J Neurol. 2011;18(11):1323-8.
232. Cordonnier C, Leys D, Dumont F, Deramecourt V, Bordet R, Pasquier F, et al. What are the causes of pre-existing dementia in patients with intracerebral haemorrhages? Brain : a journal of neurology. 2010;133(11):3281-9.
233. Harper L, Barkhof F, Scheltens P, Schott JM, Fox NC. An algorithmic approach to structural imaging in dementia. Journal of neurology, neurosurgery, and psychiatry. 2014;85(6):692-8.
234. Kalantarian S, Stern TA, Mansour M, Ruskin JN. Cognitive impairment associated with atrial fibrillation: a meta-analysis. Ann Intern Med. 2013;158(5 Pt 1):338-46.
235. Chen LY, Norby FL, Gottesman RF, Mosley TH, Soliman EZ, Agarwal SK, et al. Association of Atrial Fibrillation With Cognitive Decline and Dementia Over 20 Years: The ARIC-NCS (Atherosclerosis Risk in Communities Neurocognitive Study). Journal of the American Heart Association. 2018;7(6):e007301.
236. Kwok CS, Loke YK, Hale R, Potter JF, Myint PK. Atrial fibrillation and incidence of dementia: a systematic review and meta-analysis. Neurology. 2011;76(10):914-22.
237. de Bruijn RF, Heeringa J, Wolters FJ, Franco OH, Stricker BH, Hofman A, et al. Association Between Atrial Fibrillation and Dementia in the General Population. JAMA neurology. 2015;72(11):1288-94.
238. Singh-Manoux A, Fayosse A, Sabia S, Canonico M, Bobak M, Elbaz A, et al. Atrial fibrillation as a risk factor for cognitive decline and dementia. Eur Heart J. 2017;38(34):2612-8.
239. Santangeli P, Di Biase L, Bai R, Mohanty S, Pump A, Cereceda Brantes M, et al. Atrial fibrillation and the risk of incident dementia: a meta-analysis. Heart rhythm : the official journal of the Heart Rhythm Society. 2012;9(11):1761-8.

240. Gaita F, Corsinovi L, Anselmino M, Raimondo C, Pianelli M, Toso E, et al. Prevalence of silent cerebral ischemia in paroxysmal and persistent atrial fibrillation and correlation with cognitive function. Journal of the American College of Cardiology. 2013;62(21):1990-7.
241. Graff-Radford J, Madhavan M, Vemuri P, Rabinstein AA, Cha RH, Mielke MM, et al. Atrial fibrillation, cognitive impairment, and neuroimaging. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2016;12(4):391-8.
242. Chen LY, Lopez FL, Gottesman RF, Huxley RR, Agarwal SK, Loehr L, et al. Atrial fibrillation and cognitive decline-the role of subclinical cerebral infarcts: the atherosclerosis risk in communities study. Stroke; a journal of cerebral circulation. 2014;45(9):2568-74.
243. Gardarsdottir M, Sigurdsson S, Aspelund T, Rokita H, Launer LJ, Gudnason V, et al. Atrial fibrillation is associated with decreased total cerebral blood flow and brain perfusion. Europace. 2018;20(8):1252-8.
244. Lavy S, Stern S, Melamed E, Cooper G, Keren A, Levy P. Effect of chronic atrial fibrillation on regional cerebral blood flow. Stroke. 1980;11(1):35-8.
245. Totaro R, Corridoni C, Marini C, Marsili R, Prencipe M. Transcranial Doppler evaluation of cerebral blood flow in patients with paroxysmal atrial fibrillation. Ital J Neurol Sci. 1993;14(6):451-4.
246. Alosco ML, Spitznagel MB, Sweet LH, Josephson R, Hughes J, Gunstad J. Atrial fibrillation exacerbates cognitive dysfunction and cerebral perfusion in heart failure. Pacing Clin Electrophysiol. 2015;38(2):178-86.
247. Cacciatore F, Testa G, Langellotto A, Galizia G, Della-Morte D, Gargiulo G, et al. Role of ventricular rate response on dementia in cognitively impaired elderly subjects with atrial fibrillation: a 10-year study. Dement Geriatr Cogn Disord. 2012;34(3-4):143-8.
248. Hui DS, Morley JE, Mikolajczak PC, Lee R. Atrial fibrillation: A major risk factor for cognitive decline. American heart journal. 2015;169(4):448-56.
249. Poggesi A, Inzitari D, Pantoni L. Atrial Fibrillation and Cognition: Epidemiological Data and Possible Mechanisms. Stroke; a journal of cerebral circulation. 2015;46(11):3316-21.
250. Dietzel J, Haeusler KG, Endres M. Does atrial fibrillation cause cognitive decline and dementia? Europace. 2018;20(3):408-19.
251. Rivard L, Khairy P. Mechanisms, Clinical Significance, and Prevention of Cognitive Impairment in Patients With Atrial Fibrillation. Can J Cardiol. 2017;33(12):1556-64.

252. Knecht S, Oelschlager C, Duning T, Lohmann H, Albers J, Stehling C, et al. Atrial fibrillation in stroke-free patients is associated with memory impairment and hippocampal atrophy. Eur Heart J. 2008;29(17):2125-32.
253. Stefansdottir H, Arnar DO, Aspelund T, Sigurdsson S, Jonsdottir MK, Hjaltason H, et al. Atrial fibrillation is associated with reduced brain volume and cognitive function independent of cerebral infarcts. Stroke; a journal of cerebral circulation. 2013;44(4):1020-5.
254. Pendlebury ST. Dementia in patients hospitalized with stroke: rates, time course, and clinico-pathologic factors. International journal of stroke : official journal of the International Stroke Society. 2012;7(7):570-81.
255. van Straaten EC, Scheltens P, Knol DL, van Buchem MA, van Dijk EJ, Hofman PA, et al. Operational definitions for the NINDS-AIREN criteria for vascular dementia: an interobserver study. Stroke; a journal of cerebral circulation. 2003;34(8):1907-12.
256. Saposnik G, Cote R, Rochon PA, Mamdani M, Liu Y, Raptis S, et al. Care and outcomes in patients with ischemic stroke with and without preexisting dementia. Neurology. 2011;77(18):1664-73.
257. Wakisaka Y, Matsuo R, Hata J, Kuroda J, Kitazono T, Kamouchi M, et al. Adverse Influence of Pre-Stroke Dementia on Short-Term Functional Outcomes in Patients with Acute Ischemic Stroke: The Fukuoka Stroke Registry. Cerebrovascular diseases. 2017;43(1-2):82-9.
258. Melkas S, Oksala NK, Jokinen H, Pohjasvaara T, Vataja R, Oksala A, et al. Poststroke dementia predicts poor survival in long-term follow-up: influence of prestroke cognitive decline and previous stroke. Journal of neurology, neurosurgery, and psychiatry. 2009;80(8):865-70.
259. Henon H, Durieu I, Lebert F, Pasquier F, Leys D. Influence of prestroke dementia on early and delayed mortality in stroke patients. Journal of neurology. 2003;250(1):10-6.
260. Tang WK, Chan SS, Chiu HF, Ungvari GS, Wong KS, Kwok TC, et al. Frequency and determinants of prestroke dementia in a Chinese cohort. Journal of neurology. 2004;251(5):604-8.
261. Mok VC, Wong A, Lam WW, Fan YH, Tang WK, Kwok T, et al. Cognitive impairment and functional outcome after stroke associated with small vessel disease. Journal of neurology, neurosurgery, and psychiatry. 2004;75(4):560-6.
262. Mok V, Wong A, Tang WK, Lam WW, Fan YH, Richards PS, et al. Determinants of prestroke cognitive impairment in stroke associated with small vessel disease. Dement Geriatr Cogn Disord. 2005;20(4):225-30.

263. Henon H, Pasquier F, Durieu I, Godefroy O, Lucas C, Lebert F, et al. Preexisting dementia in stroke patients. Baseline frequency, associated factors, and outcome. Stroke; a journal of cerebral circulation. 1997;28(12):2429-36.
264. Henon H, Pasquier F, Durieu I, Pruvo JP, Leys D. Medial temporal lobe atrophy in stroke patients: relation to pre-existing dementia. Journal of neurology, neurosurgery, and psychiatry. 1998;65(5):641-7.
265. Pasquier F, Henon H, Leys D. Relevance of white matter changes to pre- and poststroke dementia. Ann N Y Acad Sci. 2000;903:466-9.
266. Pohjasvaara T, Mantyla R, Aronen HJ, Leskela M, Salonen O, Kaste M, et al. Clinical and radiological determinants of prestroke cognitive decline in a stroke cohort. Journal of neurology, neurosurgery, and psychiatry. 1999;67(6):742-8.
267. Barba R, Castro MD, del Mar Morin M, Rodriguez-Romero R, Rodriguez-Garcia E, Canton R, et al. Prestroke dementia. Cerebrovascular diseases. 2001;11(3):216-24.
268. Caratozzolo S, Riva M, Vicini Chilovi B, Cerea E, Mombelli G, Padovani A, et al. Prestroke dementia: characteristics and clinical features in consecutive series of patients. Eur Neurol. 2014;71(3-4):148-54.
269. Horstmann S, Rizos T, Rauch G, Fuchs M, Arden C, Veltkamp R. Atrial fibrillation and prestroke cognitive impairment in stroke. Journal of neurology. 2014;261(3):546-53.
270. Lefebvre C, Deplanque D, Touze E, Henon H, Parnetti L, Pasquier F, et al. Prestroke dementia in patients with atrial fibrillation. Frequency and associated factors. Journal of neurology. 2005;252(12):1504-9.
271. Pendlebury ST, Cuthbertson FC, Welch SJ, Mehta Z, Rothwell PM. Underestimation of cognitive impairment by Mini-Mental State Examination versus the Montreal Cognitive Assessment in patients with transient ischemic attack and stroke: a population-based study. Stroke; a journal of cerebral circulation. 2010;41(6):1290-3.
272. Webb AJ, Pendlebury ST, Li L, Simoni M, Lovett N, Mehta Z, et al. Validation of the Montreal cognitive assessment versus mini-mental state examination against hypertension and hypertensive arteriopathy after transient ischemic attack or minor stroke. Stroke; a journal of cerebral circulation. 2014;45(11):3337-42.
273. Wilson D, Ambler G, Shakeshaft C, Brown MM, Charidimou A, Al-Shahi Salman R, et al. Cerebral microbleeds and intracranial haemorrhage risk in patients anticoagulated for atrial fibrillation after acute ischaemic stroke or transient ischaemic attack (CROMIS-2): a multicentre observational cohort study. The Lancet Neurology. 2018;17(6):539-47.
274. Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. Journal of the American Geriatrics Society. 2005;53(4):695-9.

275. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke; a journal of cerebral circulation. 1988;19(5):604-7.
276. Uyttenboogaart M, Stewart RE, Vroomen PC, De Keyser J, Luijckx GJ. Optimizing cutoff scores for the Barthel index and the modified Rankin scale for defining outcome in acute stroke trials. Stroke; a journal of cerebral circulation. 2005;36(9):1984-7.
277. Qureshi AI, Saed A, Tasneem N, Adil MM. Neuroanatomical correlates of atrial fibrillation: a longitudinal MRI study. J Vasc Interv Neurol. 2014;7(5):18-23.
278. Seshadri S, Wolf PA, Beiser A, Elias MF, Au R, Kase CS, et al. Stroke risk profile, brain volume, and cognitive function: the Framingham Offspring Study. Neurology. 2004;63(9):1591-9.
279. Ihara M, Washida K. Linking Atrial Fibrillation with Alzheimer's Disease: Epidemiological, Pathological, and Mechanistic Evidence. J Alzheimers Dis. 2018;62(1):61-72.
280. Burton EJ, Barber R, Mukaetova-Ladinska EB, Robson J, Perry RH, Jaros E, et al. Medial temporal lobe atrophy on MRI differentiates Alzheimer's disease from dementia with Lewy bodies and vascular cognitive impairment: a prospective study with pathological verification of diagnosis. Brain : a journal of neurology. 2009;132(Pt 1):195-203.
281. Rusanen M, Kivipelto M, Levalahti E, Laatikainen T, Tuomilehto J, Soininen H, et al. Heart diseases and long-term risk of dementia and Alzheimer's disease: a population-based CAIDE study. J Alzheimers Dis. 2014;42(1):183-91.
282. Dublin S, Anderson ML, Haneuse SJ, Heckbert SR, Crane PK, Breitner JC, et al. Atrial fibrillation and risk of dementia: a prospective cohort study. Journal of the American Geriatrics Society. 2011;59(8):1369-75.
283. Bunch TJ, Weiss JP, Crandall BG, May HT, Bair TL, Osborn JS, et al. Atrial fibrillation is independently associated with senile, vascular, and Alzheimer's dementia. Heart rhythm : the official journal of the Heart Rhythm Society. 2010;7(4):433-7.
284. Dublin S, Anderson ML, Heckbert SR, Hubbard RA, Sonnen JA, Crane PK, et al. Neuropathologic changes associated with atrial fibrillation in a population-based autopsy cohort. The journals of gerontology Series A, Biological sciences and medical sciences. 2014;69(5):609-15.
285. Chen A, Akinyemi RO, Hase Y, Firbank MJ, Ndung'u MN, Foster V, et al. Frontal white matter hyperintensities, clasmatodendrosis and gliovascular abnormalities in ageing and post-stroke dementia. Brain : a journal of neurology. 2016;139(Pt 1):242-58.

286. Kalaria RN. The pathology and pathophysiology of vascular dementia. Neuropharmacology. 2018;134(Pt B):226-39.
287. Bastos-Leite AJ, van der Flier WM, van Straaten EC, Staekenborg SS, Scheltens P, Barkhof F. The contribution of medial temporal lobe atrophy and vascular pathology to cognitive impairment in vascular dementia. Stroke; a journal of cerebral circulation. 2007;38(12):3182-5.
288. Hosseini AA, Meng D, Simpson RJ, Auer DP. Mesiotemporal atrophy and hippocampal diffusivity distinguish amnestic from non-amnestic vascular cognitive impairment. Eur J Neurol. 2017;24(7):902-11.
289. Haeusler KG, Wilson D, Fiebach JB, Kirchhof P, Werring DJ. Brain MRI to personalise atrial fibrillation therapy: current evidence and perspectives. Heart. 2014;100(18):1408-13.
290. Mayasi Y, Helenius J, McManus DD, Goddeau RP, Jr., Jun-O'Connell AH, Moonis M, et al. Atrial fibrillation is associated with anterior predominant white matter lesions in patients presenting with embolic stroke. Journal of neurology, neurosurgery, and psychiatry. 2018;89(1):6-13.
291. Mok VC, Lam BY, Wong A, Ko H, Markus HS, Wong LK. Early-onset and delayed-onset poststroke dementia - revisiting the mechanisms. Nat Rev Neurol. 2017;13(3):148-59.
292. Mok VCT, Lam BYK, Wang Z, Liu W, Au L, Leung EYL, et al. Delayed-onset dementia after stroke or transient ischemic attack. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2016;12(11):1167-76.
293. Altieri M, Di Piero V, Pasquini M, Gasparini M, Vanacore N, Vicenzini E, et al. Delayed poststroke dementia: a 4-year follow-up study. Neurology. 2004;62(12):2193-7.
294. Hurford R, Charidimou A, Fox Z, Cipolotti L, Werring DJ. Domain-specific trends in cognitive impairment after acute ischaemic stroke. Journal of neurology. 2013;260(1):237-41.
295. Ballard C, Rowan E, Stephens S, Kalaria R, Kenny RA. Prospective follow-up study between 3 and 15 months after stroke: improvements and decline in cognitive function among dementia-free stroke survivors >75 years of age. Stroke; a journal of cerebral circulation. 2003;34(10):2440-4.
296. Douiri A, Rudd AG, Wolfe CD. Prevalence of poststroke cognitive impairment: South London Stroke Register 1995-2010. Stroke; a journal of cerebral circulation. 2013;44(1):138-45.
297. Hochstenbach JB, den Otter R, Mulder TW. Cognitive recovery after stroke: a 2-year follow-up. Arch Phys Med Rehabil. 2003;84(10):1499-504.

298. Patel M, Coshall C, Rudd AG, Wolfe CD. Natural history of cognitive impairment after stroke and factors associated with its recovery. Clin Rehabil. 2003;17(2):158-66.
299. Sivakumar L, Kate M, Jeerakathil T, Camicioli R, Buck B, Butcher K. Serial montreal cognitive assessments demonstrate reversible cognitive impairment in patients with acute transient ischemic attack and minor stroke. Stroke; a journal of cerebral circulation. 2014;45(6):1709-15.
300. Nijsse B, Visser-Meily JM, van Mierlo ML, Post MW, de Kort PL, van Heugten CM. Temporal Evolution of Poststroke Cognitive Impairment Using the Montreal Cognitive Assessment. Stroke; a journal of cerebral circulation. 2017;48(1):98-104.
301. Yoon JA, Kim DY, Sohn MK, Lee J, Lee SG, Lee YS, et al. Factors associated with improvement or decline in cognitive function after an ischemic stroke in Korea: the Korean stroke cohort for functioning and rehabilitation (KOSCO) study. BMC Neurol. 2017;17(1):9.
302. Tang WK, Chen YK, Lu JY, Wong A, Mok V, Chu WC, et al. Absence of cerebral microbleeds predicts reversion of vascular 'cognitive impairment no dementia' in stroke. International journal of stroke : official journal of the International Stroke Society. 2011;6(6):498-505.
303. Renou P, Sibon I, Tourdias T, Rouanet F, Rosso C, Galanaud D, et al. Reliability of the ECASS radiological classification of postthrombolysis brain haemorrhage: a comparison of CT and three MRI sequences. Cerebrovascular diseases. 2010;29(6):597-604.
304. Gregoire SM, Scheffler G, Jager HR, Yousry TA, Brown MM, Kallis C, et al. Strictly lobar microbleeds are associated with executive impairment in patients with ischemic stroke or transient ischemic attack. Stroke; a journal of cerebral circulation. 2013;44(5):1267-72.
305. Chan E, Altendorff S, Healy C, Werring DJ, Cipolotti L. The test accuracy of the Montreal Cognitive Assessment (MoCA) by stroke lateralisation. Journal of the neurological sciences. 2017;373:100-4.
306. Chan E, Khan S, Oliver R, Gill SK, Werring DJ, Cipolotti L. Underestimation of cognitive impairments by the Montreal Cognitive Assessment (MoCA) in an acute stroke unit population. Journal of the neurological sciences. 2014;343(1-2):176-9.
307. Bhalla A, Wang Y, Rudd A, Wolfe CD. Differences in outcome and predictors between ischemic and intracerebral hemorrhage: the South London Stroke Register. Stroke; a journal of cerebral circulation. 2013;44(8):2174-81.
308. Bejot Y, Blanc C, Delpont B, Thouant P, Chazalon C, Daumas A, et al. Increasing early ambulation disability in spontaneous intracerebral hemorrhage survivors. Neurology. 2018;90(23):e2017-e24.

309. Moulin S, Cordonnier C. Prognosis and Outcome of Intracerebral Haemorrhage. Front Neurol Neurosci. 2015;37:182-92.
310. Paroutoglou K, Parry-Jones AR. Hyperacute management of intracerebral haemorrhage. Clin Med (Lond). 2018;18(Suppl 2):s9-s12.
311. Sprigg N, Flaherty K, Appleton JP, Al-Shahi Salman R, Bereczki D, Beridze M, et al. Tranexamic acid for hyperacute primary IntraCerebral Haemorrhage (TICH-2): an international randomised, placebo-controlled, phase 3 superiority trial. Lancet. 2018;391(10135):2107-15.
312. Eckman MH, Rosand J, Knudsen KA, Singer DE, Greenberg SM. Can patients be anticoagulated after intracerebral hemorrhage? A decision analysis. Stroke; a journal of cerebral circulation. 2003;34(7):1710-6.
313. Crook R, Hardy J, Duff K. Single-day apolipoprotein E genotyping. Journal of neuroscience methods. 1994;53(2):125-7.
314. HES Data Dictionary: Admitted Patient Care 2017. Available from: https://digital.nhs.uk/data-and-information/data-tools-and-services/data-services/hospital-episode-statistics/hospital-episode-statistics-data-dictionary.
315. World Health Organisation International Statistical Classification of Diseases and Related Health Problems 10th Revision World Health Organisation; [updated 23/04/2018. Available from: http://apps.who.int/classifications/icd10/browse/2016/en.
316. van Swieten JC, Hijdra A, Koudstaal PJ, van Gijn J. Grading white matter lesions on CT and MRI: a simple scale. J Neurol Neurosurg Psychiatry. 1990;53(12):1080-3.
317. Specogna AV, Turin TC, Patten SB, Hill MD. Factors associated with early deterioration after spontaneous intracerebral hemorrhage: a systematic review and meta-analysis. PloS one. 2014;9(5):e96743.
318. Rost NS, Smith EE, Chang Y, Snider RW, Chanderraj R, Schwab K, et al. Prediction of functional outcome in patients with primary intracerebral hemorrhage: the FUNC score. Stroke; a journal of cerebral circulation. 2008;39(8):2304-9.
319. Hemphill JC, 3rd, Bonovich DC, Besmertis L, Manley GT, Johnston SC. The ICH score: a simple, reliable grading scale for intracerebral hemorrhage. Stroke; a journal of cerebral circulation. 2001;32(4):891-7.
320. Cheung RT, Zou LY. Use of the original, modified, or new intracerebral hemorrhage score to predict mortality and morbidity after intracerebral hemorrhage. Stroke; a journal of cerebral circulation. 2003;34(7):1717-22.
321. Godoy DA, Pinero G, Di Napoli M. Predicting mortality in spontaneous intracerebral hemorrhage: can modification to original score improve the prediction? Stroke; a journal of cerebral circulation. 2006;37(4):1038-44.

322. Weimar C, Roth M, Willig V, Kostopoulos P, Benemann J, Diener HC. Development and validation of a prognostic model to predict recovery following intracerebral hemorrhage. Journal of neurology. 2006;253(6):788-93.
323. Ruiz-Sandoval JL, Chiquete E, Romero-Vargas S, Padilla-Martinez JJ, Gonzalez-Cornejo S. Grading scale for prediction of outcome in primary intracerebral hemorrhages. Stroke; a journal of cerebral circulation. 2007;38(5):1641-4.
324. Cho DY, Chen CC, Lee WY, Lee HC, Ho LH. A new Modified Intracerebral Hemorrhage score for treatment decisions in basal ganglia hemorrhage--a randomized trial. Crit Care Med. 2008;36(7):2151-6.
325. Chuang YC, Chen YM, Peng SK, Peng SY. Risk stratification for predicting 30-day mortality of intracerebral hemorrhage. Int J Qual Health Care. 2009;21(6):441-7.
326. Parry-Jones AR, Paley L, Bray BD, Hoffman AM, James M, Cloud GC, et al. Care-limiting decisions in acute stroke and association with survival: analyses of UK national quality register data. International journal of stroke : official journal of the International Stroke Society. 2016;11(3):321-31.
327. Zahuranec DB, Brown DL, Lisabeth LD, Gonzales NR, Longwell PJ, Smith MA, et al. Early care limitations independently predict mortality after intracerebral hemorrhage. Neurology. 2007;68(20):1651-7.
328. Wilson D, Charidimou A, Shakeshaft C, Ambler G, White M, Cohen H, et al. Volume and functional outcome of intracerebral hemorrhage according to oral anticoagulant type. Neurology. 2016;86(4):360-6.
329. Vermeer SE, Algra A, Franke CL, Koudstaal PJ, Rinkel GJ. Long-term prognosis after recovery from primary intracerebral hemorrhage. Neurology. 2002;59(2):205-9.
330. Fogelholm R, Murros K, Rissanen A, Avikainen S. Long term survival after primary intracerebral haemorrhage: a retrospective population based study. Journal of neurology, neurosurgery, and psychiatry. 2005;76(11):1534-8.
331. Koivunen RJ, Tatlisumak T, Satopaa J, Niemela M, Putaala J. Intracerebral hemorrhage at young age: long-term prognosis. Eur J Neurol. 2015;22(7):1029-37.
332. Tveiten A, Ljostad U, Mygland A, Naess H. Leukoaraiosis is associated with short- and long-term mortality in patients with intracerebral hemorrhage. J Stroke Cerebrovasc Dis. 2013;22(7):919-25.
333. Hansen BM, Nilsson OG, Anderson H, Norrving B, Saveland H, Lindgren A. Long term (13 years) prognosis after primary intracerebral haemorrhage: a prospective population based study of long term mortality, prognostic factors and causes of death. Journal of neurology, neurosurgery, and psychiatry. 2013;84(10):1150-5.
334. Flaherty ML, Haverbusch M, Sekar P, Kissela B, Kleindorfer D, Moomaw CJ, et al. Long-term mortality after intracerebral hemorrhage. Neurology. 2006;66(8):1182-6.

335. Palm F, Henschke N, Wolf J, Zimmer K, Safer A, Schroder RJ, et al. Intracerebral haemorrhage in a population-based stroke registry (LuSSt): incidence, aetiology, functional outcome and mortality. Journal of neurology. 2013;260(10):2541-50.
336. Lane CA, Parker TD, Cash DM, Macpherson K, Donnachie E, Murray-Smith H, et al. Study protocol: Insight 46 - a neuroscience sub-study of the MRC National Survey of Health and Development. BMC Neurol. 2017;17(1):75.
337. Elrod JK, Fortenberry JL, Jr. The hub-and-spoke organization design: an avenue for serving patients well. BMC Health Serv Res. 2017;17(Suppl 1):457.
338. Tamura R, Tomita H, Mizutani K, Miwa T. The importance of amyloid beta protein in cerebrospinal fluid when you recognize convexal subarachnoid hemorrhage. Eur Neurol. 2014;71(5-6):283-7.
339. Garcia Estevez DA, Garcia-Dorrego RM, Nieto-Baltar B, Marey-Garrido M, Hierro-Torner T. Spontaneous convexity subarachnoid haemorrhage: Clinical series of 3 patients with associated cerebral amyloid angiopathy. Neurologia. 2017;32(4):213-8.
340. Martinez-Lizana E, Carmona-Iragui M, Alcolea D, Gomez-Choco M, Vilaplana E, Sanchez-Saudinos MB, et al. Cerebral amyloid angiopathy-related atraumatic convexal subarachnoid hemorrhage: an ARIA before the tsunami. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2015;35(5):710-7.
341. Shams S, Granberg T, Martola J, Li X, Shams M, Fereshtehnejad SM, et al. Cerebrospinal fluid profiles with increasing number of cerebral microbleeds in a continuum of cognitive impairment. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2016;36(3):621-8.
342. Gentil BJ, Tibshirani M, Durham HD. Neurofilament dynamics and involvement in neurological disorders. Cell Tissue Res. 2015;360(3):609-20.
343. Zetterberg H. Neurofilament Light: A Dynamic Cross-Disease Fluid Biomarker for Neurodegeneration. Neuron. 2016;91(1):1-3.
344. Rosengren LE, Karlsson JE, Sjogren M, Blennow K, Wallin A. Neurofilament protein levels in CSF are increased in dementia. Neurology. 1999;52(5):1090-3.
345. de Jong D, Jansen RW, Pijnenburg YA, van Geel WJ, Borm GF, Kremer HP, et al. CSF neurofilament proteins in the differential diagnosis of dementia. Journal of neurology, neurosurgery, and psychiatry. 2007;78(9):936-8.
346. Skillback T, Farahmand B, Bartlett JW, Rosen C, Mattsson N, Nagga K, et al. CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. Neurology. 2014;83(21):1945-53.

347. Disanto G, Barro C, Benkert P, Naegelin Y, Schadelin S, Giardiello A, et al. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol. 2017;81(6):857-70.
348. Kuhle J, Barro C, Disanto G, Mathias A, Soneson C, Bonnier G, et al. Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult Scler. 2016;22(12):1550-9.
349. De Marchis GM, Katan M, Barro C, Fladt J, Traenka C, Seiffge DJ, et al. Serum neurofilament light chain in patients with acute cerebrovascular events. Eur J Neurol. 2018;25(3):562-8.
350. Hjalmarsson C, Bjerke M, Andersson B, Blennow K, Zetterberg H, Aberg ND, et al. Neuronal and glia-related biomarkers in cerebrospinal fluid of patients with acute ischemic stroke. Journal of central nervous system disease. 2014;6:51-8.
351. Traenka C, Disanto G, Seiffge DJ, Gensicke H, Hert L, Grond-Ginsbach C, et al. Serum Neurofilament Light Chain Levels Are Associated with Clinical Characteristics and Outcome in Patients with Cervical Artery Dissection. Cerebrovascular diseases. 2015;40(5-6):222-7.
352. Norgren N, Rosengren L, Stigbrand T. Elevated neurofilament levels in neurological diseases. Brain Res. 2003;987(1):25-31.
353. Jonsson M, Zetterberg H, van Straaten E, Lind K, Syversen S, Edman A, et al. Cerebrospinal fluid biomarkers of white matter lesions - cross-sectional results from the LADIS study. Eur J Neurol. 2010;17(3):377-82.
354. Gattringer T, Pinter D, Enzinger C, Seifert-Held T, Kneihsl M, Fandler S, et al. Serum neurofilament light is sensitive to active cerebral small vessel disease. Neurology. 2017;89(20):2108-14.
355. Duering M, Konieczny MJ, Tiedt S, Baykara E, Tuladhar AM, Leijsen EV, et al. Serum Neurofilament Light Chain Levels Are Related to Small Vessel Disease Burden. Journal of stroke. 2018;20(2):228-38.
356. Ulrich JD, Ulland TK, Colonna M, Holtzman DM. Elucidating the Role of TREM2 in Alzheimer's Disease. Neuron. 2017;94(2):237-48.
357. Jin SC, Benitez BA, Karch CM, Cooper B, Skorupa T, Carrell D, et al. Coding variants in TREM2 increase risk for Alzheimer's disease. Human molecular genetics. 2014;23(21):5838-46.
358. Gispert JD, Suarez-Calvet M, Monte GC, Tucholka A, Falcon C, Rojas S, et al. Cerebrospinal fluid sTREM2 levels are associated with gray matter volume increases and reduced diffusivity in early Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2016;12(12):1259-72.

359. Heslegrave A, Heywood W, Paterson R, Magdalinou N, Svensson J, Johansson P, et al. Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. Mol Neurodegener. 2016;11:3.
360. Piccio L, Deming Y, Del-Aguila JL, Ghezzi L, Holtzman DM, Fagan AM, et al. Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status. Acta neuropathologica. 2016;131(6):925-33.
361. Suarez-Calvet M, Kleinberger G, Araque Caballero MA, Brendel M, Rominger A, Alcolea D, et al. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. EMBO Mol Med. 2016;8(5):466-76.
362. Suarez-Calvet M, Araque Caballero MA, Kleinberger G, Bateman RJ, Fagan AM, Morris JC, et al. Early changes in CSF sTREM2 in dominantly inherited Alzheimer's disease occur after amyloid deposition and neuronal injury. Sci Transl Med. 2016;8(369):369ra178.
363. Piccio L, Buonsanti C, Cella M, Tassi I, Schmidt RE, Fenoglio C, et al. Identification of soluble TREM-2 in the cerebrospinal fluid and its association with multiple sclerosis and CNS inflammation. Brain : a journal of neurology. 2008;131(Pt 11):3081-91.
364. Ohrfelt A, Axelsson M, Malmestrom C, Novakova L, Heslegrave A, Blennow K, et al. Soluble TREM-2 in cerebrospinal fluid from patients with multiple sclerosis treated with natalizumab or mitoxantrone. Mult Scler. 2016;22(12):1587-95.
365. Portelius E, Zetterberg H, Skillback T, Tornqvist U, Andreasson U, Trojanowski JQ, et al. Cerebrospinal fluid neurogranin: relation to cognition and neurodegeneration in Alzheimer's disease. Brain : a journal of neurology. 2015;138(Pt 11):3373-85.
366. Portelius E, Olsson B, Hoglund K, Cullen NC, Kvartsberg H, Andreasson U, et al. Cerebrospinal fluid neurogranin concentration in neurodegeneration: relation to clinical phenotypes and neuropathology. Acta neuropathologica. 2018;136(3):363-76.
367. Kester MI, Teunissen CE, Crimmins DL, Herries EM, Ladenson JH, Scheltens P, et al. Neurogranin as a Cerebrospinal Fluid Biomarker for Synaptic Loss in Symptomatic Alzheimer Disease. JAMA neurology. 2015;72(11):1275-80.
368. Kvartsberg H, Duits FH, Ingelsson M, Andreasen N, Ohrfelt A, Andersson K, et al. Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2015;11(10):1180-90.
369. Mattsson N, Insel PS, Palmqvist S, Portelius E, Zetterberg H, Weiner M, et al. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. EMBO Mol Med. 2016;8(10):1184-96.

370. Sanfilippo C, Forlenza O, Zetterberg H, Blennow K. Increased neurogranin concentrations in cerebrospinal fluid of Alzheimer's disease and in mild cognitive impairment due to AD. J Neural Transm (Vienna). 2016;123(12):1443-7.
371. Tarawneh R, D'Angelo G, Crimmins D, Herries E, Griest T, Fagan AM, et al. Diagnostic and Prognostic Utility of the Synaptic Marker Neurogranin in Alzheimer Disease. JAMA neurology. 2016;73(5):561-71.
372. Wellington H, Paterson RW, Portelius E, Tornqvist U, Magdalinou N, Fox NC, et al. Increased CSF neurogranin concentration is specific to Alzheimer disease. Neurology. 2016;86(9):829-35.
373. Wellington H, Paterson RW, Suarez-Gonzalez A, Poole T, Frost C, Sjobom U, et al. CSF neurogranin or tau distinguish typical and atypical Alzheimer disease. Annals of clinical and translational neurology. 2018;5(2):162-71.
374. Casaletto KB, Elahi FM, Bettcher BM, Neuhaus J, Bendlin BB, Asthana S, et al. Neurogranin, a synaptic protein, is associated with memory independent of Alzheimer biomarkers. Neurology. 2017;89(17):1782-8.
375. Lista S, Toschi N, Baldacci F, Zetterberg H, Blennow K, Kilimann I, et al. Cerebrospinal Fluid Neurogranin as a Biomarker of Neurodegenerative Diseases: A Cross-Sectional Study. J Alzheimers Dis. 2017;59(4):1327-34.
376. Headley A, De Leon-Benedetti A, Dong C, Levin B, Loewenstein D, Camargo C, et al. Neurogranin as a predictor of memory and executive function decline in MCI patients. Neurology. 2018;90(10):e887-e95.
377. Wang L, Alzheimer's Disease Neuroimaging I. Association of cerebrospinal fluid Neurogranin with Alzheimer's disease. Aging Clin Exp Res. 2019;31(2):185-91.
378. De Vos A, Bjerke M, Brouns R, De Roeck N, Jacobs D, Van den Abbeele L, et al. Neurogranin and tau in cerebrospinal fluid and plasma of patients with acute ischemic stroke. BMC Neurol. 2017;17(1):170.
379. Ayton S, Diouf I, Bush AI, Alzheimer's disease Neuroimaging I. Evidence that iron accelerates Alzheimer's pathology: a CSF biomarker study. Journal of neurology, neurosurgery, and psychiatry. 2018;89(5):456-60.
380. Ayton S, Faux NG, Bush AI, Alzheimer's Disease Neuroimaging I. Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE. Nature communications. 2015;6:6760.
381. Renard D, Thouvenot E. CSF RBC count in successful first-attempt lumbar puncture: the interest of atraumatic needle use. Neurol Sci. 2017;38(12):2189-93.
382. Elahi FM, Miller BL. A clinicopathological approach to the diagnosis of dementia. Nat Rev Neurol. 2017;13(8):457-76.
383. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol. 2010;6(3):131-44.

384. Thompson AGB, Luk C, Heslegrave AJ, Zetterberg H, Mead SH, Collinge J, et al. Neurofilament light chain and tau concentrations are markedly increased in the serum of patients with sporadic Creutzfeldt-Jakob disease, and tau correlates with rate of disease progression. Journal of neurology, neurosurgery, and psychiatry. 2018;89(9):955-61.
385. Kleinberger G, Yamanishi Y, Suarez-Calvet M, Czirr E, Lohmann E, Cuyvers E, et al. TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. Sci Transl Med. 2014;6(243):243ra86.
386. Keir G, Tasdemir N, Thompson EJ. Cerebrospinal fluid ferritin in brain necrosis: evidence for local synthesis. Clin Chim Acta. 1993;216(1-2):153-66.
387. Albargothy NJ, Johnston DA, MacGregor-Sharp M, Weller RO, Verma A, Hawkes CA, et al. Convective influx/glymphatic system: tracers injected into the CSF enter and leave the brain along separate periarterial basement membrane pathways. Acta neuropathologica. 2018;136(1):139-52.
388. Kummer MP, Heneka MT. Truncated and modified amyloid-beta species. Alzheimers Res Ther. 2014;6(3):28.
389. Selkoe DJ. The molecular pathology of Alzheimer's disease. Neuron. 1991;6(4):487-98.
390. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992;256(5054):184-5.
391. Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). Neuron. 1994;13(1):45-53.
392. Boche D, Zotova E, Weller RO, Love S, Neal JW, Pickering RM, et al. Consequence of Abeta immunization on the vasculature of human Alzheimer's disease brain. Brain : a journal of neurology. 2008;131(Pt 12):3299-310.
393. Sperling RA, Jack CR, Jr., Black SE, Frosch MP, Greenberg SM, Hyman BT, et al. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: recommendations from the Alzheimer's Association Research Roundtable Workgroup. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2011;7(4):367-85.
394. Arrighi HM, Barakos J, Barkhof F, Tampieri D, Jack C, Jr., Melancon D, et al. Amyloid-related imaging abnormalities-haemosiderin (ARIA-H) in patients with Alzheimer's disease treated with bapineuzumab: a historical, prospective secondary analysis. Journal of neurology, neurosurgery, and psychiatry. 2016;87(1):106-12.
395. Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci. 2007;8(9):663-72.
396. Ittner A, Ittner LM. Dendritic Tau in Alzheimer's Disease. Neuron. 2018;99(1):13-27.

397. Matute-Blanch C, Villar LM, Alvarez-Cermeno JC, Rejdak K, Evdoshenko E, Makshakov G, et al. Neurofilament light chain and oligoclonal bands are prognostic biomarkers in radiologically isolated syndrome. Brain : a journal of neurology. 2018;141(4):1085-93.
398. Barro C, Benkert P, Disanto G, Tsagkas C, Amann M, Naegelin Y, et al. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain : a journal of neurology. 2018;141(8):2382-91.
399. Novakova L, Axelsson M, Khademi M, Zetterberg H, Blennow K, Malmestrom C, et al. Cerebrospinal fluid biomarkers as a measure of disease activity and treatment efficacy in relapsing-remitting multiple sclerosis. J Neurochem. 2017;141(2):296-304.
400. Villemagne VL, Rowe CC. Long night's journey into the day: amyloid-beta imaging in Alzheimer's disease. J Alzheimers Dis. 2013;33 Suppl 1:S349-59.
401. Villemagne VL, Dore V, Burnham SC, Masters CL, Rowe CC. Imaging tau and amyloid-beta proteinopathies in Alzheimer disease and other conditions. Nat Rev Neurol. 2018;14(4):225-36.
402. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol. 2004;55(3):306-19.
403. Herholz K, Ebmeier K. Clinical amyloid imaging in Alzheimer's disease. The Lancet Neurology. 2011;10(7):667-70.
404. Vinters HV, Gilbert JJ. Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes. Stroke; a journal of cerebral circulation. 1983;14(6):924-8.
405. Vinters HV, Zarow C, Borys E, Whitman JD, Tung S, Ellis WG, et al. Review: Vascular dementia: clinicopathologic and genetic considerations. Neuropathol Appl Neurobiol. 2018;44(3):247-66.
406. Kovari E, Herrmann FR, Hof PR, Bouras C. The relationship between cerebral amyloid angiopathy and cortical microinfarcts in brain ageing and Alzheimer's disease. Neuropathol Appl Neurobiol. 2013;39(5):498-509.
407. Revesz T, Holton JL, Lashley T, Plant G, Rostagno A, Ghiso J, et al. Sporadic and familial cerebral amyloid angiopathies. Brain Pathol. 2002;12(3):343-57.
408. Yamada M, Tsukagoshi H, Otomo E, Hayakawa M. Cerebral amyloid angiopathy in the aged. Journal of neurology. 1987;234(6):371-6.
409. Pantelakis S. [A particular type of senile angiopathy of the central nervous system: congophilic angiopathy, topography and frequency]. Monatsschr Psychiatr Neurol. 1954;128(4):219-56.
410. Attems J, Quass M, Jellinger KA, Lintner F. Topographical distribution of cerebral amyloid angiopathy and its effect on cognitive decline are influenced by

Alzheimer disease pathology. Journal of the neurological sciences. 2007;257(1-2):49-55.
411. Thal DR, Ghebremedhin E, Rub U, Yamaguchi H, Del Tredici K, Braak H. Two types of sporadic cerebral amyloid angiopathy. J Neuropathol Exp Neurol. 2002;61(3):282-93.
412. Lockhart A, Lamb JR, Osredkar T, Sue LI, Joyce JN, Ye L, et al. PIB is a non-specific imaging marker of amyloid-beta (Abeta) peptide-related cerebral amyloidosis. Brain : a journal of neurology. 2007;130(Pt 10):2607-15.
413. Bacskai BJ, Klunk WE, Mathis CA, Hyman BT. Imaging amyloid-beta deposits in vivo. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2002;22(9):1035-41.
414. Bacskai BJ, Frosch MP, Freeman SH, Raymond SB, Augustinack JC, Johnson KA, et al. Molecular imaging with Pittsburgh Compound B confirmed at autopsy: a case report. Arch Neurol. 2007;64(3):431-4.
415. Ossenkoppele R, Jansen WJ, Rabinovici GD, Knol DL, van der Flier WM, van Berckel BN, et al. Prevalence of amyloid PET positivity in dementia syndromes: a meta-analysis. Jama. 2015;313(19):1939-49.
416. Burgos N, Cardoso MJ, Modat M, Punwani S, Atkinson D, Arridge SR, et al. CT synthesis in the head & neck region for PET/MR attenuation correction: an iterative multi-atlas approach. EJNMMI physics. 2015;2(Suppl 1):A31.
417. Ladefoged CN, Law I, Anazodo U, St Lawrence K, Izquierdo-Garcia D, Catana C, et al. A multi-centre evaluation of eleven clinically feasible brain PET/MRI attenuation correction techniques using a large cohort of patients. Neuroimage. 2017;147:346-59.
418. Erlandsson K, Buvat I, Pretorius PH, Thomas BA, Hutton BF. A review of partial volume correction techniques for emission tomography and their applications in neurology, cardiology and oncology. Phys Med Biol. 2012;57(21):R119-59.
419. Modat M, Cash DM, Daga P, Winston GP, Duncan JS, Ourselin S. Global image registration using a symmetric block-matching approach. Journal of medical imaging (Bellingham, Wash). 2014;1(2):024003.
420. Cardoso MJ, Modat M, Wolz R, Melbourne A, Cash D, Rueckert D, et al. Geodesic Information Flows: Spatially-Variant Graphs and Their Application to Segmentation and Fusion. IEEE Trans Med Imaging. 2015;34(9):1976-88.
421. Landau SM, Lu M, Joshi AD, Pontecorvo M, Mintun MA, Trojanowski JQ, et al. Comparing positron emission tomography imaging and cerebrospinal fluid measurements of beta-amyloid. Ann Neurol. 2013;74(6):826-36.
422. Joshi AD, Pontecorvo MJ, Clark CM, Carpenter AP, Jennings DL, Sadowsky CH, et al. Performance characteristics of amyloid PET with florbetapir F 18 in patients

with alzheimer's disease and cognitively normal subjects. J Nucl Med. 2012;53(3):378-84.
423. Landau SM, Mintun MA, Joshi AD, Koeppe RA, Petersen RC, Aisen PS, et al. Amyloid deposition, hypometabolism, and longitudinal cognitive decline. Ann Neurol. 2012;72(4):578-86.
424. Gauthier S, Albert M, Fox N, Goedert M, Kivipelto M, Mestre-Ferrandiz J, et al. Why has therapy development for dementia failed in the last two decades? Alzheimer's & dementia : the journal of the Alzheimer's Association. 2016;12(1):60-4.
425. Anderson RM, Hadjichrysanthou C, Evans S, Wong MM. Why do so many clinical trials of therapies for Alzheimer's disease fail? Lancet. 2017;390(10110):2327-9.
426. Kantarci K, Gunter JL, Tosakulwong N, Weigand SD, Senjem MS, Petersen RC, et al. Focal hemosiderin deposits and beta-amyloid load in the ADNI cohort. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2013;9(5 Suppl):S116-23.
427. Zonneveld HI, Goos JD, Wattjes MP, Prins ND, Scheltens P, van der Flier WM, et al. Prevalence of cortical superficial siderosis in a memory clinic population. Neurology. 2014;82(8):698-704.
428. Wollenweber FA, Buerger K, Mueller C, Ertl-Wagner B, Malik R, Dichgans M, et al. Prevalence of cortical superficial siderosis in patients with cognitive impairment. Journal of neurology. 2014;261(2):277-82.
429. Inoue Y, Nakajima M, Uetani H, Hirai T, Ueda M, Kitajima M, et al. Diagnostic Significance of Cortical Superficial Siderosis for Alzheimer Disease in Patients with Cognitive Impairment. AJNR Am J Neuroradiol. 2016;37(2):223-7.
430. Charidimou A, Ni J, Martinez-Ramirez S, Vashkevich A, Ayres A, Rosand J, et al. Cortical Superficial Siderosis in Memory Clinic Patients: Further Evidence for Underlying Cerebral Amyloid Angiopathy. Cerebrovascular diseases. 2016;41(3-4):156-62.
431. Vernooij MW, Ikram MA, Hofman A, Krestin GP, Breteler MM, van der Lugt A. Superficial siderosis in the general population. Neurology. 2009;73(3):202-5.
432. Catafau AM, Bullich S, Seibyl JP, Barthel H, Ghetti B, Leverenz J, et al. Cerebellar Amyloid-beta Plaques: How Frequent Are They, and Do They Influence 18F-Florbetaben SUV Ratios? J Nucl Med. 2016;57(11):1740-5.
433. Itoh Y, Yamada M, Hayakawa M, Otomo E, Miyatake T. Cerebral amyloid angiopathy: a significant cause of cerebellar as well as lobar cerebral hemorrhage in the elderly. Journal of the neurological sciences. 1993;116(2):135-41.

434. Boyle PA, Yu L, Wilson RS, Leurgans SE, Schneider JA, Bennett DA. Person-specific contribution of neuropathologies to cognitive loss in old age. Ann Neurol. 2018;83(1):74-83.
435. Arvanitakis Z, Capuano AW, Leurgans SE, Bennett DA, Schneider JA. Relation of cerebral vessel disease to Alzheimer's disease dementia and cognitive function in elderly people: a cross-sectional study. The Lancet Neurology. 2016;15(9):934-43.

Appendix 1: CROMIS-2 Collaborators

Louise Shaw, MD, Kirsty Harkness MD, Jane Sword, MD, Azlisham Mohd Nor, MD, Pankaj Sharma, PhD, Deborah Kelly, MD, Frances Harrington, MD, Marc Randall, MD, Matthew Smith, MD, Karim Mahawish, MD, Abduelbaset Elmarim, MD, Bernard Esisi, MD, Claire Cullen, MD, Arumug Nallasivam, MD, Christopher Price, MD, Adrian Barry, MD, Christine Roffe, MD, John Coyle, MD, Ahamad Hassan, MD, Caroline Lovelock, DPhil, Jonathan Birns, MD, David Cohen, MD, L Sekaran, MD, Adrian Parry-Jones, PhD, Anthea Parry, MD, David Hargroves, MD, Harald Proschel, MD, Prabel Datta, MD, Khaled Darawil, MD, Aravindakshan Manoj, MD, Mathew Burn, MD, Chris Patterson, MD, Elio Giallombardo, MD, Nigel Smyth, MD, Syed Mansoor, MD, Ijaz Anwar, MD, Rachel Marsh, MD, Sissi Ispoglou, MD, Dinesh Chadha, MD, Mathuri Prabhakaran, MD, Sanjeevikumar Meenakishundaram, MD, Janice O'Connell, MD, Jon Scott, MD, Vinodh Krishnamurthy, MD, Prasanna Aghoram, MD, Michael McCormick, MD, Paul O'Mahony, MD, Martin Cooper, MD, Lillian Choy, MD, Peter Wilkinson, MD, Simon Leach, MD, Sarah Caine, MD, Ilse Burger, MD, Gunaratam Gunathilagan, MD, Paul Guyler, MD, Hedley Emsley, MD, Michelle Davis, MD, Dulka Manawadu, MD, Kath Pasco, MD, Maam Mamun, MD, Robert Luder, MD, Mahmud Sajid, MD, Ijaz Anwar, MD, James Okwera, MD, Julie Staals, PhD, Elizabeth Warburton, MD, Kari Saastamoinen, MD, Timothy England, MD, Janet Putterill, MD, Enrico Flossman, MD, Michael Power, MD, Krishna Dani, MD, David Mangion, MD, Appu Suman, MD, John Corrigan, MD, Enas Lawrence, MD, and Djamil Vahidassr, MD.

Appendix 2: Publications

Full list of publications to which the candidate has made a contribution during her time as a PhD student. * indicates joint authorship

1. **Banerjee G**, Wilson D, Jäger HR, Werring DJ. Novel imaging techniques in cerebral small vessel diseases and vascular cognitive impairment. *Biochim Biophys Acta* 2016; 1862(5):926-38. PubMed PMID: 26687324.
2. **Banerjee G**, Wahab KW, Gregoire SM, Jichi F, Charidimou A, Jäger HR, Rantell K, Werring DJ. Impaired renal function is related to deep and mixed, but not strictly lobar cerebral microbleeds in patients with ischaemic stroke and TIA. *J Neurol* 2016; 263(4):760-4. PubMed PMID: 26886202.
3. Werring D, **Banerjee G**. Cerebral Amyloid Angiopathy and Intracerebral Haemorrhage. *ACNR* 2016; 16(2): 8-12.
4. Wilson D, Hostettler IC, Ambler G, **Banerjee G**, Jäger HR, Werring DJ. Convexity subarachnoid haemorrhage has a high risk of intracerebral haemorrhage in suspected cerebral amyloid angiopathy. *J Neurol* 2017; 264(4):674. PubMed PMID: 28243752.
5. **Banerjee G**, Kim HJ, Fox Z, Jäger HR, Wilson D, Charidimou A, Na HK, Na DL, Seo SW, Werring DJ. MRI-visible perivascular space location is associated with Alzheimer's disease independently of amyloid burden. *Brain* 2017; 140(4):1107-1116. PubMed PMID: 28335021.
6. **Banerjee G**, Carare R, Cordonnier C, Greenberg SM, Schneider JA, Smith EE, Buchem MV, Grond JV, Verbeek MM, Werring DJ. The increasing impact of cerebral amyloid angiopathy: essential new insights for clinical practice. *J Neurol Neurosurg Psychiatry* 2017; 88(11):982-994. PubMed PMID: 28844070.
7. **Banerjee G**, Wilson D, Ambler G, Osei-Bonsu Appiah K, Shakeshaft C, Lunawat S, Cohen H, Yousry T, Lip GYH, Muir KW, Brown MM, Al-Shahi Salman R, Jäger HR, Werring DJ; on behalf of the CROMIS-2 collaborators. Cognitive impairment before

intracerebral haemorrhage is associated with cerebral amyloid angiopathy. *Stroke* 2018; 49(1): 40-45. PubMed PMID: 29247143.

8. **Banerjee G***, Jang H*, Kim HJ, Kim ST, Kim JS, Lee JH, Im K, Kwon H, Lee JM, Na DL, Seo SW, Werring DJ. Total MRI Small Vessel Disease Burden Correlates with Cognitive Performance, Cortical Atrophy, and Network Measures in a Memory Clinic Population. *J Alzheimers Dis*. 2018; 63(4):1485-1497. PubMed PMID: 29843234.
9. **Banerjee G**, Alvares D, Bowen J, Adams ME, Werring DJ. Minimally symptomatic cerebral amyloid angiopathy-related inflammation: three descriptive case reports. *J Neurol Neurosurg Psychiatry*. 2018. PubMed PMID: 29535144.
10. Wilson D, Ambler G, Shakeshaft C, Brown MM, Charidimou A, Al-Shahi Salman R, Lip GYH, Cohen H, **Banerjee G**, Houlden H, White MJ, Yousry TA, Harkness K, Flossmann E, Smyth N, Shaw LJ, Warburton E, Muir KW, Jäger HR, Werring DJ; on behalf of the CROMIS-2 collaborators. Cerebral microbleeds and intracranial haemorrhage risk in patients anticoagulated for atrial fibrillation after acute ischaemic stroke or transient ischaemic attack (CROMIS-2): a multicentre observational cohort study. *Lancet Neurol*. 2018; 17(6): 539-547. PubMed PMID: 29778365.
11. **Banerjee G***, Summers M*, Chan E, Wilson D, Charidimou A, Cipolotti L, Werring DJ. Domain-specific characterisation of early cognitive impairment following spontaneous intracerebral haemorrhage. *J Neurol Sci*. 2018; 391:25-30. PubMed PMID: 30103965.
12. Ziff, OJ, **Banerjee G**, Ambler G, Werring DJ. Statins and the risk of intracerebral haemorrhage in patients with stroke: systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry.* 2018. PubMed PMID: 30150320.

Submitted:

I. **Banerjee** G, Adams ME, Jaunmuktane Z, Lammie GA, Turner B, Wani M, Sawhney IMS, Houlden H, Mead S, Brandner S, Werring DJ. Early-onset cerebral amyloid angiopathy following childhood exposure to cadaveric dura. Submitted to *Ann Neurol*.

II. **Banerjee G**, Chan E, Ambler G, Wilson D, Cipolotti L, Shakeshaft C, Cohen H, Yousry TA, Al-Shahi Salman R, Lip GYH, Brown MM, Muir KW, Jäger HR, Werring DJ; on behalf of the CROMIS-2 collaborators. Cognitive impairment prior to atrial fibrillation related ischaemic events: neuroimaging and prognostic associations. Submitted to *J Neurol Neurosurg Psychiatry*.

III. **Banerjee G**, Chan E, Ambler G, Wilson D, Cipolotti L, Shakeshaft C, Cohen H, Yousry TA, Lip GYH, Brown MM, Muir KW, Jäger HR, Werring DJ; on behalf of the CROMIS-2 collaborators. Effect of small vessel disease on cognitive trajectory after atrial fibrillation related ischaemic stroke or TIA. Submitted to *Stroke*.

IV. Jensen MP, Ziff OJ, **Banerjee G**, Ambler G, Werring DJ. The impact of selective serotonin reuptake inhibitors on the risk of intracranial haemorrhage: a systematic review and meta-analysis. Submitted to *European Stroke Journal*.

V. Wilson D, Ambler G, **Banerjee G**, Shakeshaft C, Cohen H, Yousry TA, Al-Shahi Salman R, Lip GYH, Houlden H, Brown MM, Muir KW, Jäger HR, Werring DJ; on behalf of the CROMIS-2 collaborators. Early versus late anticoagulation for ischaemic stroke associated with atrial fibrillation: multicenter cohort study. Submitted to *J Neurol Neurosurg Psychiatry.*

VI. Wilson D, Ambler G, Hostettler IC, **Banerjee G**, Shakeshaft C, Cohen H, Yousry TA, Al-Shahi Salman R, Lip GYH, Houlden H, Brown MM, Muir KW, Jäger HR, Werring DJ; on behalf of the CROMIS-2 collaborators. Risk of recurrent intracerebral hemorrhage and ischemic stroke after intracerebral hemorrhage: multicentre prospective cohort study. Submitted to *Neurology*.

Appendix 3: BOCAA Study Documents and Forms

I. Standard Operating Procedure

Abbreviations:

AC	Ana Carvalho (INM)
AT	Alice Tucker (LWENC CRF)
CRF	Case Report Form
CSF	Cerebrospinal fluid
DB	David Brown (INM)
DJW	Professor David J Werring (Principle Investigator)
EC	Dr Edgar Chan (Department of Neuropsychology);
GB	Dr Gargi Banerjee (Study Co-ordinator)
HVIS	Healthy Volunteer Information Sheet
INM	Institute of Nuclear Medicine
LC	Professor Lisa Cipolotti (Department of Neuropsychology)
LP	Lumbar puncture
LWENC CRF	Leonard Wolfson Experimental Neurology Centre Clinical Research Facility
PIS	Patient Information Sheet
SRN	Stroke Research Nurse
UCLH	University College London Hospital

Patient selection / initial approach:

☐ DJW and GB to review imaging of potential participants and ensure eligibility for trial
☐ Potential participants to be contacted by post (invitation letter / PIS / HVIS); subsequent contact (if participant agrees) by email / telephone

Prior to research visit, once participant has agreed to take part in study:

☐ GB to contact AT and submit booking form for participant **screening visit** (to take place at LWENC CRF)
☐ At screening visit, GB to complete **screening checklist** to ensure eligibility, and then **consent participant**
☐ GB to send letter to participant confirming appointment date and details

☐ GB to contact INM (DB ± AC) and confirm PET-MR date and time slot for participant
☐ GB to submit study PET-MR request form
☐ GB to contact AT and submit booking form for participant (visit 1 and 2)
☐ AT to confirm dates for participant's Visits 1 and 2; ***NOTE VISIT 2 MUST TAKE PLACE WITHIN TWO WEEKS OF VISIT 1***
☐ AT to generate UCLH hospital number (if necessary) for participant
☐ AT to generate/retrieve notes for participant
☐ GB to inform Department of Neuropsychology (EC) of date for participant's Visit 1, and confirm time
☐ GB to submit Department of Neuropsychology request form (EC/LC)
☐ GB to contact participant (email/telephone) in order to confirm appointment date and details
☐ GB to send letter to participant confirming appointment date and details

Visit 1 (full day):

☐ Participant arrival at LWENC CRF by 8.30AM; they must be fasted
☐ Participant to have fasting blood samples taken by LWENC CRF staff at LWENC CRF (please see separate Blood Sample Checklist / SOP for full details)
☐ Patient to have breakfast
☐ CRF and Participant Information Sheet to be completed by GB / LWENC staff

☐ Neuropsychological testing to start at approx. 10.30AM (EC; contact details above); LWENC CRF staff to walk participant to Department of Neuropsychology
☐ Once neuropsychological testing complete (estimate by 1PM); participant lunch / short break
☐ LWENC CRF staff to walk participant from LWENC CRF to University College Hospital Macmillan Cancer Centre, Huntley Street, for PET-MR scan appointment (appointments likely to be from 2.30PM onwards)
☐ Participant to have PET-MR scan
☐ END OF VISIT 1 FOR PARTICIPANT; GB to bring notes back to LWENC CRF in preparation for Visit 2

☐ GB to review blood results and imaging results once available; any unexpected abnormalities to be immediately communicated to participant, DJW, participant GP and LWENC CRF
☐ GB to post letter to participant GP to inform them of study involvement

Visit 2 (half day):
☐ VISIT 2 MUST TAKE PLACE WITHIN TWO WEEKS OF VISIT 1
☐ Participant arrival at LWENC CRF for LP
☐ LP to be performed by LWENC CRF staff (please see separate CSF Sample Checklist / SOP for full details)
☐ END OF VISIT 2 FOR PARTICIPANT

Follow up:
☐ GB/SRN to post follow up questionnaire to patient and patient GP at 6 months, 1 year, and then annually (for up to 5 years)

II. Patient Information Sheet

You are invited to participate in a research project in which we are collecting information to try and find new markers for the disease Cerebral Amyloid Angiopathy (CAA). Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and any other doctors if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

Small blood vessels (less than a millimetre or so in diameter) supply the brain with blood. Diseases of these small blood vessels become very common with increasing age. Cerebral amyloid angiopathy (CAA) is one of these "small vessel diseases", and affects 25% of those over the age of 70. It is associated with a type of stroke called spontaneous intracerebral haemorrhage (ICH) which is bleeding within the brain). ICH is responsible for 10% of stroke in high-income countries and 20% in low-income and middle-income countries. CAA can also cause difficulties with memory and cognition.

CAA has recently become a promising target for treatment, but we still don't fully understand why some people get CAA, or why it causes strokes and memory problems in some people. More research is needed before we can properly evaluate new treatments. One way of approaching these questions is by identifying new "biomarkers". Biomarkers are measures that can tell us a variety of things; sometimes they can tell us whether someone has a disease or not, sometimes they can tell us how severe a disease is, and sometimes they can tell us about how a disease is likely to progress. If a biomarker could tell us about disease severity or progression, we could use it to test future treatment – if the biomarker got better, we could see that our treatment was working.

This project aims to find new biomarkers for CAA. It will do this by looking for differences between patients with CAA and healthy people of a similar age who do not have CAA. One way of detecting differences will be by comparing brain scans between these two groups. This project will use a new type of scan protocol that allows two types of scan to take place at the same time. These two types of scan are called Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET). The PET part of the scan will

use a specific compound called florbetapir (or "Amyvid"). This project will also look for differences in the fluid that surrounds the brain and spinal cord, which is called cerebrospinal fluid, or CSF. The project will check other measures, such as levels of patient disability and tests of memory and attention (as examples), and check to see whether any of the new biomarkers correlate with these measures.

Why have I been chosen?

You have been chosen because you have CAA.

Do I have to take part?

Your participation in the study is entirely voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to stop taking part at any time and without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

If you agree to join the study, we will collect your medical details from you and your medical notes. You will initially have a brief clinical assessment, during which you will have a couple of short memory and attention tests, and during which you will be asked questions about your daily life and medical history; this is to ensure that you are eligible to take part in the study. You will receive the best possible medical care and have the usual investigations including brain scans that form part of normal patient care. You will receive £20 towards covering your travel costs.

You will have a blood sample taken for routine tests and also for genetic and other tests. The routine tests are to ensure that it is safe to perform a lumbar puncture. The other tests will look at genes or substances in the blood that may be related to the presence of CAA. You will not receive any results from the research genetic or other blood tests. Should the research discover genetic or other blood tests of clinical significance, all participants will be contacted and asked if they wish to have further investigations performed.

You will also have neuropsychological testing. This will be carried out by a trained clinical psychologist, who will ask you to perform a number of tasks that will test lots of brain functions, including your memory, language skills and attention. This testing will take approximately 60 minutes, and will not involve any physically invasive tasks. There will also be some questions about your daily activities, and a short test of walking speed, where you will have to stand up from a chair, walk a specified distance, turn around and return to the chair.

You will have a PET-MRI scan lasting approximately 55 minutes; you may have had a similar scan done before. You will receive an injection of a radioactive tracer (florbetapir, or "Amyvid") once you are in the scanner – the MRI and PET scans will then take place simultaneously. You will lie down flat in a small tunnel, and will hear some knocking and buzzing noises. We can offer you some earplugs to make this noise less unpleasant for you.

You will have a lumbar puncture, which is a procedure in which a sample of cerebrospinal fluid (the fluid that surrounds the brain and spinal cord) is taken. This will then be tested for biomarkers. The test will be performed by a doctor trained in this procedure. A needle is inserted into the lower part of the spine (after some local anaesthetic to numb the area), and samples are collected via this needle. The procedure is usually carried out whilst you are lying on your side, with your legs pulled up and your chin tucked in; in some situations, the procedure may be carried out whilst you are sat up and leaning forwards. The whole process is likely to last approximately 30 minutes.

All of these tests except the lumbar puncture will be completed during a single day. The lumbar puncture will take place within approximately two weeks of your first visit. The reason for this delay is to allow us to check your blood tests, and to ensure enough time between the lumbar puncture and the PET-MRI scan to avoid any interference between the two. Having the lumbar puncture on another day will also allow you to have time to recover afterwards.

You will be sent a questionnaire at 6 and 12 months, which will ask questions about your general health. You may also be sent further similar questionnaires annually, up to five years after taking part in the study.

We will write to your GP to inform them that you are enrolled in this study, if you choose to take part. We may also contact your GP in the future with regard to changes in your health status.

The data from this study will be used to contribute towards an educational qualification. The data from this project may be shared with other research groups, both within the UK and internationally. If this is the case, the data will be anonymised, which means that nobody would be able to identify you from it.

You will not routinely receive any results from the research tests. Should any of these tests identify results of clinical significance, you will be contacted and asked if you wish to have further investigations performed. Your GP will also be contacted and informed of any unexpectedly abnormal results.

What are the possible benefits of taking part?

This project will provide access to a study team with expertise in CAA, providing participants with more information regarding their disease and its prognosis. This study will be important in identifying new biomarkers for CAA, and could lead to CAA being identified more accurately and at an earlier stage, as well as correlating these markers with clinical outcomes. This study will be essential in informing future therapeutic studies, which will benefit future patients with CAA. This is especially relevant as currently there are no treatments for CAA. More broadly, the study will be of benefit to patient support groups and disease specific charities (such as the Stroke Association), which will be in a better position to educate patients, their families and carers, together with the general public and policy makers about CAA.

What are the possible disadvantages of taking part?

You may experience minor discomfort or anxiety during blood taking, but this will be minimised according to standard clinical procedures and by ensuring only appropriately trained personnel undertake the testing.

The MRI scan is noisy and may provoke claustrophobia; noise is minimised using earplugs, anxiety and claustrophobia are reduced by continuous communication during the scan via intercom.

Neuropsychological testing may similarly cause minor inconvenience.

The PET scan involves exposure to radiation, and any exposure to radiation has a risk of inducing cancer. However, the radiation dose used in a PET scan is very low and extremely unlikely to be hazardous to your health. The total radiation dose you will be exposed to is 7.0 mSv - this will confer a risk for lifetime mortality from cancer of about 1 in 4,900. This estimate should be compared with the lifetime natural incidence rate of cancer in the UK, which is approximately 1 in 3. The extra risk of cancer due to this exposure represents an increase in this natural rate of incidence in the UK population of under 0.1%. At the mean UK background radiation rate of 2.4 mSv per year, this effective radiation dose of 7.0 mSv also represents the equivalent of just under three years exposure to natural background radiation. Parts of the UK, e.g. Cornwall, experience background radiation approximately three times higher than this due to the increased concentration of radon of geological origin − thus the 7.0 mSv effective dose stated above will equate to approximately one year of exposure at this enhanced natural background level. Although the level of radiation from the PET scan is small, we advise that you avoid close contact with children and pregnant women for the rest of the day; this may be inconvenient for you. In addition, if you are planning to travel abroad in the near future you may trigger one of the very sensitive radiation detectors located at airports, train stations or seaports. In the unlikely event that this occurs there is no need to be alarmed. Customs officials will understand what has happened, however, we recommend you carry your appointment letter with you as proof of your recent test.

A lumbar puncture is generally safe and the risk of serious complications is low. Common side effects include localised swelling, back pain or rash; these usually settle within a couple of days. Another common side effect is headache, which is thought to be due to a persistent slow leak of spinal fluid from the lumbar puncture site. This “post-lumbar puncture headache” may affect up to 40% of people. These headaches are typically worse when in the upright position and are relieved by lying down. They usually resolve within hours, and drinking plenty of fluids and taking simple painkillers will help. If the headache persists for more than 2 days then you may require a procedure called a blood patch. This would require blood to be taken from your arm and injected into the site of the lumbar puncture in order to seal the leak. A study in a population similar to this one reported post-lumbar puncture headache in 9% of participants; only 0.3% of all those studied required a blood patch. Significant risks include infection or bleeding at the site

of needle entry, and nerve damage; these risks are rare. All risks will be minimised by using standard clinical procedures (including use of local anaesthetic), and by ensuring only appropriately trained personnel undertake the testing. You will be asked to sign a written consent form prior to this procedure, and the doctor will discuss the procedure once again including its potential risks at this time.

All staff on the study will be fully trained in good clinical practice, and will always consider the well-being of the participant as their primary concern. You can withdraw from the study at any time without affecting your clinical care.

What happens if something goes wrong?

Every care will be taken in the course of this study. However, in the unlikely event that you are injured by taking part, compensation may be available. If you suspect that the injury is the result of negligence then you may be able to claim compensation. Injury that results as a consequence of the design or management of the research is covered by the Sponsor (Joint Research Office, University College London). If you suspect that the injury is the result of negligence on the part of the hospital or a hospital employee, this is covered by NHS or relevant professional indemnity respectively.

After discussing with your research doctor, please make the claim in writing to Dr David Werring who is the Chief Investigator for the research and is based at UCL Institute of Neurology. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's Office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff or about any side effects (adverse events) you may have experienced due to your participation in the research, the normal National Health Service complaints mechanisms are available to you. Please ask your research doctor if you would like more information on this. Details can also be obtained from the Department of Health website: http://www.dh.gov.uk.

Will my taking part in this study be kept confidential?

All information regarding your medical records will be treated as strictly confidential and will only be used for medical research on CAA. The medical information will be kept at

the UCL Stroke Research Centre, UCL Institute of Neurology at Russell Square House (London, UK) for analysis. Professor David Werring, the Chief Investigator, will be responsible for the security and access to the information. The data may be used for future research on stroke by UCL and/or other research institutions in the UK but your confidentiality will be strictly maintained. Your medical records may be inspected by competent authorities and properly authorized persons, but if any information is released outside the trial office it will be transferred in a secure manner. The results of the study will be published in medical journals or other public sites.

If you want to take out life insurance, health insurance or a mortgage, companies may ask you about any genetic tests that you may have had. We keep research results confidential.

In summary, if you take part, you will have:

- A short initial assessment
- A blood sample collected for a routine tests as well as for genetic analysis
- Neuropsychological testing and functional assessment
- An PET-MRI scan
- A lumbar puncture
- Questionnaires sent to you at 6 months and 12 months after testing (and annually up to five years after testing)

Thank you for reading this information sheet and taking the time to consider participating in this study. If you agree to take part, you will be given a copy of this information sheet and a copy of the signed consent form.

Further information can be obtained from:

Professor David Werring, Consultant Neurologist
[redacted]
Tel: [redacted]; email: [redacted]

Dr Gargi Banerjee, BOCAA Study Co-ordinator
[redacted]
Tel: [redacted]; email: [redacted]

III. Healthy Volunteer Information Sheet

You are invited to participate in a research project in which we are collecting information to try and find new markers for the disease Cerebral Amyloid Angiopathy (CAA). You have been invited as a healthy volunteer, which means that you do not have the disease. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and any other doctors if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

Small blood vessels (less than a millimetre or so in diameter) supply the brain with blood. Diseases of these small blood vessels become very common with increasing age. Cerebral amyloid angiopathy (CAA) is one of these "small vessel diseases", and affects 25% of those over the age of 70. It is associated with a type of stroke called spontaneous intracerebral haemorrhage (ICH) which is bleeding within the brain). ICH is responsible for 10% of stroke in high-income countries and 20% in low-income and middle-income countries. CAA can also cause difficulties with memory and cognition.

CAA has recently become a promising target for treatment, but we still don't fully understand why some people get CAA, or why it causes strokes and memory problems in some people. More research is needed before we can properly evaluate new treatments. One way of approaching these questions is by identifying new "biomarkers". Biomarkers are measures that can tell us a variety of things; sometimes they can tell us whether someone has a disease or not, sometimes they can tell us how severe a disease is, and sometimes they can tell us about how a disease is likely to progress. If a biomarker could tell us about disease severity or progression, we could use it to test future treatment – if the biomarker got better, we could see that our treatment was working.

This project aims to find new biomarkers for CAA. It will do this by looking for differences between patients with CAA and healthy people of a similar age who do not have CAA. One way of detecting differences will be by comparing brain scans between these two groups. This project will use a new type of scan protocol that allows two types of scan to take place at the same time. These two types of scan are called Magnetic Resonance

Imaging (MRI) and Positron Emission Tomography (PET). The PET part of the scan will use a specific compound called florbetapir (or "Amyvid"). This project will also look for differences in the fluid that surrounds the brain and spinal cord, which is called cerebrospinal fluid, or CSF. The project will check other measures, such as levels of patient disability and tests of memory and attention (as examples), and check to see whether any of the new biomarkers correlate with these measures.

Why have I been chosen?

You have been chosen as a healthy volunteer because you do not have CAA, and are of the correct age.

Do I have to take part?

Your participation in the study is entirely voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to stop taking part at any time and without giving a reason.

What will happen to me if I take part?

If you agree to join the study, we will collect your medical details from you. You will initially have a brief clinical assessment, during which you will have a couple of short memory and attention tests, and during which you will be asked questions about your daily life and medical history; this is to ensure that you are eligible to take part in the study. You will receive £20 towards covering your travel costs.

You will have a blood sample taken for routine tests and also for genetic and other tests. The routine tests are to ensure that it is safe to perform a lumbar puncture. The other tests will look at genes or substances in the blood that may be related to the presence of CAA.

You will also have neuropsychological testing. This will be carried out by a trained clinical psychologist, who will ask you to perform a number of tasks that will test lots of brain functions, including your memory, language skills and attention. This testing will take approximately 60 minutes, and will not involve any physically invasive tasks. There will also be some questions about your daily activities, and a short test of walking speed,

where you will have to stand up from a chair, walk a specified distance, turn around and return to the chair.

You will have a PET-MRI scan lasting approximately 55 minutes; you may have had a similar scan done before. You will receive an injection of a radioactive tracer (florbetapir, or "Amyvid") once you are in the scanner – the MRI and PET scans will then take place simultaneously. You will lie down flat in a small tunnel, and will hear some knocking and buzzing noises. We can offer you some earplugs to make this noise less unpleasant for you.

You will have a lumbar puncture, which is a procedure in which a sample of cerebrospinal fluid (the fluid that surrounds the brain and spinal cord) is taken. This will then be tested for biomarkers. The test will be performed by a doctor trained in this procedure. A needle is inserted into the lower part of the spine (after some local anaesthetic to numb the area), and samples are collected via this needle. The procedure is usually carried out whilst you are lying on your side, with your legs pulled up and your chin tucked in; in some situations, the procedure may be carried out whilst you are sat up and leaning forwards. The whole process is likely to last approximately 30 minutes.

All of these tests except the lumbar puncture will be completed during a single day. The lumbar puncture will take place within approximately two weeks of your first visit. The reason for this delay is to allow us to check your blood tests, and to ensure enough time between the lumbar puncture and the PET-MRI scan to avoid any interference between the two. Having the lumbar puncture on another day will also allow you to have time to recover afterwards.

We will write to your GP to inform them that you are enrolled in this study, if you choose to take part. We may also contact your GP in the future with regard to changes in your health status.

The data from this study will be used to contribute towards an educational qualification. The data from this project may be shared with other research groups, both within the UK and internationally. If this is the case, the data will be anonymised, which means that nobody would be able to identify you from it.

You will not routinely receive any results from the research tests. Should any of these tests identify results of clinical significance, you will be contacted and asked if you wish to have further investigations performed. Your GP will also be contacted and informed of any unexpectedly abnormal results.

What are the possible benefits of taking part?

As a healthy volunteer, there is no benefit to taking part, as you do not have CAA.

What are the possible disadvantages of taking part?

You may experience minor discomfort or anxiety during blood taking, but this will be minimised according to standard clinical procedures and by ensuring only appropriately trained personnel undertake the testing.

Neuropsychological testing may similarly cause minor inconvenience.

The MRI scan is noisy and may provoke claustrophobia; noise is minimised using earplugs, anxiety and claustrophobia are reduced by continuous communication during the scan via intercom.

The PET scan involves exposure to radiation, and any exposure to radiation has a risk of inducing cancer. However, the radiation dose used in a PET scan is very low and extremely unlikely to be hazardous to your health. The total radiation dose you will be exposed to is 7.0 mSv - this will confer a risk for lifetime mortality from cancer of about 1 in 4,900. This estimate should be compared with the lifetime natural incidence rate of cancer in the UK, which is approximately 1 in 3. The extra risk of cancer due to this exposure represents an increase in this natural rate of incidence in the UK population of under 0.1%. At the mean UK background radiation rate of 2.4 mSv per year, this effective radiation dose of 7.0 mSv also represents the equivalent of just under three years exposure to natural background radiation. Parts of the UK, e.g. Cornwall, experience background radiation approximately three times higher than this due to the increased concentration of radon of geological origin − thus the 7.0 mSv effective dose stated above will equate to approximately one year of exposure at this enhanced natural background level. Although the level of radiation from the PET scan is small, we advise that you avoid close contact with children and pregnant women for the rest of the day;

this may be inconvenient for you. In addition, if you are planning to travel abroad in the near future you may trigger one of the very sensitive radiation detectors located at airports, train stations or seaports. In the unlikely event that this occurs there is no need to be alarmed. Customs officials will understand what has happened, however, we recommend you carry your appointment letter with you as proof of your recent test.

A lumbar puncture is generally safe and the risk of serious complications is low. Common side effects include localised swelling, back pain or rash; these usually settle within a couple of days. Another common side effect is headache, which is thought to be due to a persistent slow leak of spinal fluid from the lumbar puncture site. This “post-lumbar puncture headache” may affect up to 40% of people. These headaches are typically worse when in the upright position and are relieved by lying down. They usually resolve within hours, and drinking plenty of fluids and taking simple painkillers will help. If the headache persists for more than 2 days then you may require a procedure called a blood patch. This would require blood to be taken from your arm and injected into the site of the lumbar puncture in order to seal the leak. A study in a population similar to this one reported post-lumbar puncture headache in 9% of participants; only 0.3% of all those studied required a blood patch. Significant risks include infection or bleeding at the site of needle entry, and nerve damage; these risks are rare. All risks will be minimised by using standard clinical procedures (including use of local anaesthetic), and by ensuring only appropriately trained personnel undertake the testing. You will be asked to sign a written consent form prior to this procedure, and the doctor will discuss the procedure once again including its potential risks at this time.

All staff on the study will be fully trained in good clinical practice, and will always consider the well-being of the participant as their primary concern. You can withdraw from the study at any time.

What happens if something goes wrong?

Every care will be taken in the course of this study. However, in the unlikely event that you are injured by taking part, compensation may be available. If you suspect that the injury is the result of negligence then you may be able to claim compensation. Injury that results as a consequence of the design or management of the research is covered by the Sponsor (Joint Research Office, University College London). If you suspect that the injury is the result of negligence on the part of the hospital or a hospital employee, this is covered by NHS or relevant professional indemnity respectively.

After discussing with your research doctor, please make the claim in writing to Dr David Werring who is the Chief Investigator for the research and is based at UCL Institute of Neurology. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's Office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff or about any side effects (adverse events) you may have experienced due to your participation in the research, the normal National Health Service complaints mechanisms are available to you. Please ask your research doctor if you would like more information on this. Details can also be obtained from the Department of Health website: http://www.dh.gov.uk.

Will my taking part in this study be kept confidential?

All information regarding your medical records will be treated as strictly confidential and will only be used for medical research. The medical information will be kept at the UCL Stroke Research Centre, UCL Institute of Neurology at Russell Square House (London, UK) for analysis. Professor David Werring, the Chief Investigator, will be responsible for the security and access to the information. The data may be used for future research on stroke by UCL and/or other research institutions in the UK but your confidentiality will be strictly maintained. The medical records generated by this project may be inspected by competent authorities and properly authorized persons, but if any information is released outside the trial office it will be transferred in a secure manner. The results of the study will be published in medical journals or other public sites.

If you want to take out life insurance, health insurance or a mortgage, companies may ask you about any genetic tests that you may have had. We keep research results confidential.

In summary, if you take part, you will have:

- A short initial assessment
- A blood sample collected for a routine tests as well as for genetic analysis
- Neuropsychological testing and functional assessment
- An PET-MRI scan
- A lumbar puncture

Thank you for reading this information sheet and taking the time to consider participating in this study. If you agree to take part, you will be given a copy of this information sheet and a copy of the signed consent form.

Further information can be obtained from:

Professor David Werring, Consultant Neurologist

[redacted]

Tel: [redacted]; email: [redacted]

Dr Gargi Banerjee, BOCAA Study Co-ordinator

[redacted]

Tel: [redacted]; email: [redacted]

IV. Screening Checklist

Biomarkers and Outcomes in CAA (BOCAA)

Screening Checklist

Study ID	
Date of completion	DD / MM / YYYY
Name of person completing screening checklist	
Signature	

PATIENT ELIGIBILITY

Inclusion Criteria	Yes	No
Age 55 – 100 years (inclusive)		
MMSE ≥ 23 *(see appendix 1)*		
mRS ≤ 3 *(see appendix 2)*		
At least “probable” Modified Boston Criteria for CAA		
Competent to give informed consent		

If any of the above are checked “No”, the patient is **NOT** eligible for the study – **STOP**.

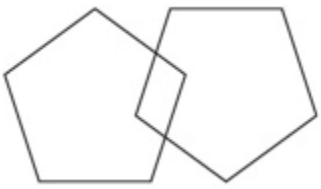
Exclusion Criteria	Yes	No
Contraindications to MRI scanning: ☐ Pacemaker or defibrillator ☐ Metallic foreign bodies within eyes ☐ Deep brain stimulator ☐ Bullets or gunshot pellets ☐ Cerebral aneurysm clips ☐ Cochlear implant ☐ Other MRI-incompatible implants or devices		
Contraindications to lumbar puncture: ☐ Possible raised intracranial pressure ☐ Ongoing anticoagulant therapy ☐ Thrombocytopenia or other bleeding diathesis ☐ Suspected spinal epidural abscess		
Contraindications to PET scanning: ☐ Pregnancy ☐ Breastfeeding ☐ Unavoidable close contact with young children ☐ Recent chemotherapy or radiotherapy		

If any of the above are checked “Yes”, the patient is **NOT** eligible for the study – **STOP**.

CURRENT MEDICATIONS

Drug Name	Dose	Frequency

Appendix 1: The Mini-Mental State Exam (MMSE)
Adapted from Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-98.

	Score	Maximum points
ORIENTATION	**1 point per correct response**	**10**
What is the year?		5
What is the season?		
What is the date?		
What is the day?		
What is the month?		
Where are we? Which state?		5
Which country?		
Which town?		
Which hospital?		
Which floor?		
REGISTRATION	**1 point per correct response**	**3**
Name 3 objects; then ask the patient to repeat them back to you		3
ATTENTION AND CALCULATION	**1 point per correct response**	**5**
Serial 7s: ask the patient to start at 100 and then take away 7; stop after five answers.		5
Alternative: spell "world" backwards.		*5*
RECALL	**1 point per correct response**	**3**
Ask for the 3 objects repeated above.		3
LANGUAGE	**1 point per correct response**	**9**
Name a pencil and a watch		2
Repeat the following: "No ifs, ands, or buts"		1
Follow a three stage command e.g. "take this paper in your hand, fold it in half and put it on the floor"		3
Read and obey the following: **"CLOSE YOUR EYES"**		1
Write a sentence		1
Copy this design: 		1
TOTAL		**30**

Appendix 2: The Modified Rankin Scale (mRS)

Score	Description
0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties and activities
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6	Dead

V. Patient Consent Form

Participant Study Number: ☐☐☐☐☐

Please complete in BLACK ball point pen

Observational study of Biomarkers and Outcomes in Cerebral Amyloid Angiopathy (BOCAA)

CONSENT FORM

Please initial boxes below

Chief Investigator: Professor David Werring

1. I confirm that I have read and understood the Patient Information Sheet **(V2.0, dated 29 September 2015)** for the above study and have had the opportunity to ask questions. I understand that the data in this study will be included in future related clinical research projects. ☐

2. I confirm that I have had sufficient time to consider whether or not I want to be included in the study. ☐

3. 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. ☐

4. I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

5. I agree to have a blood sample taken both for routine tests and for a genetic test as part of the study protocol. I understand this is given as a gift. I understand that the sample will be stored for analysis of biomarkers and for use in genetic research related to the trial and future related clinical research projects. ☐

6. I agree to have a cerebrospinal fluid sample taken as part of the study protocol. I understand that I will complete a further written consent with regard to this procedure. I understand this is given as a gift. I understand that the sample will be stored for analysis of biomarkers and for use in genetic research related to the trial and future related clinical research projects. ☐

7. I agree to research being carried out on tissue samples taken as part of my clinical care. ☐

8. I agree to my GP being informed about my taking part in this study and my GP will be sent a copy of this consent form and the information leaflet. I give permission to the researcher to contact my GP if they are any unexpected findings in my cerebrospinal fluid or blood tests. I agree to my GP being contacted in the future for updates regarding my health status. ☐

9. I understand that information held by the NHS and records maintained by the NHS Information Centre (IC) may be used to keep in touch with me and follow up my health status. ☐

10. I understand that information from this study may be shared with other collaborating centres in an anonymised form (i.e. in which I will not be identifiable). ☐

11. I agree to being contacted in the future with regard to potential follow up once this study has ended. ☐

12. I agree to take part in the above study. ☐

	☐☐☐☐☐☐	
Name of participant (please PRINT)	Date (DD/MM/YY)	Signature of participant
	☐☐☐☐☐☐	
Name of person taking consent (please PRINT)	Date (DD/MM/YY)	Signature of person taking consent

One copy to be given to the participant; one copy to be kept with hospital notes, original copy to the site data collection file

VI. Healthy Volunteer Consent Form

Participant Study Number: ☐☐☐☐☐

Please complete in BLACK ball point pen

Please initial boxes below

Chief Investigator: Professor David Werring

1. I confirm that I have read and understood the Healthy Volunteer Information Sheet **(V2.0, dated 29 September 2015)** for the above study and have had the opportunity to ask questions. I understand that the data in this study will be included in future related clinical research projects. ☐

2. I confirm that I have had sufficient time to consider whether or not I want to be included in the study. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time. ☐

4. I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

5. I agree to have a blood sample taken both for routine tests and for a genetic test as part of the study protocol. I understand this is given as a gift. I understand that the sample will be stored for analysis of biomarkers and for use in genetic research related to the trial and future related clinical research projects. ☐

6. I agree to have a cerebrospinal fluid sample taken as part of the study protocol. I understand that I will complete a further written consent with regard to this procedure. I understand this is given as a gift. I understand that the sample will be stored for analysis of biomarkers and for use in genetic research related to the trial and future related clinical research projects. ☐

7. I agree to my GP being informed about my taking part in this study and my GP will be sent a copy of this consent form and the information leaflet. I give permission to the researcher to contact my GP if they are any unexpected findings in my cerebrospinal fluid or blood tests. I agree to my GP being contacted in the future for updates regarding my health status. ☐

8. I understand that information held by the NHS and records maintained by the NHS Information Centre (IC) may be used to keep in touch with me and follow up my health status. ☐

9. I understand that information from this study may be shared with other collaborating centres in an anonymised form (i.e. in which I will not be identifiable). ☐

10. I agree to being contacted in the future with regard to potential follow up once this study has ended. ☐

11. I agree to take part in the above study. ☐

	☐☐☐☐☐☐	
Name of participant (please PRINT)	Date (DD/MM/YY)	Signature of participant

	☐☐☐☐☐☐	
Name of person taking consent (please PRINT)	Date (DD/MM/YY)	Signature of person taking consent

One copy to be given to the participant; one copy to be kept with hospital notes, original copy to the site data collection file

VII. Participant Information

BOCAA: PARTICIPANT INFORMATION

Hospital ID			
NHS Number			
Date of Birth	DD / MM / YYYY		
Surname			
First name(s)			
Sex	Male	Female	
Hand preference	Left	Right	Unknown
Address			
Contact telephone number			

Next of kin (NOK)	
Relationship to participant	
NOK address	
NOK contact telephone number	

GP name	
GP address	
GP telephone number	

VIII. Patient Case Report Form

Biomarkers and Outcomes in CAA (BOCAA)

Case Report Form (CRF)

Please fill in this form for each participant in **BLOCK CAPITALS** and **BLACK INK**.
Please enter the participant's unique study number on each page.

Participant Initials	
Study ID	

Date of CRF completion	DD / MM / YYYY
Name of person completing CRF	
Signature	

PAST MEDICAL HISTORY

Condition	Yes	No	If yes, please provide date(s) of diagnosis.
Hypertension			
Hypercholesterolaemia			
Diabetes Mellitus Type 1			
Diabetes Mellitus Type 2			
Myocardial Infarction			
Cardiac revascularisation (either PCI or CABG)			
Congestive heart failure			
Angina			
Atrial fibrillation			
Peripheral vascular disease			
Dementia or other cognitive impairment			
Seizures			
Migraine with aura			
Migraine without aura			
Transient focal neurological episodes			
Previous ischaemic stroke			
Previous TIA			
Previous ICH			

Please use this space to provide details of any other conditions or procedures. Please include dates where possible.	

Abbreviations:

CABG	Coronary Artery Bypass Grafting
ICH	Intracerebral haemorrhage
PCI	Percutaneous Coronary Intervention
TIA	Transient Ischaemic Attack

CURRENT MEDICATIONS

Drug Name	Dose	Frequency

Any Allergies?

SOCIAL HISTORY			
	Yes	**No**	**If yes, please provide further details below.**
Does the patient currently smoke?			Number of cigarettes per day: If the patient smokes tobacco in another form, e.g. pipes, roll ups, etc., please provide details below (method and quantity per day).
If the patient is currently a non-smoker, have they previously ever smoked?			Year started: Year stopped: Number of cigarettes per day:
Does the patient currently drink alcohol?			Number of units per week:
If the patient currently does not drink alcohol, have they done so previously?			Year started: Year stopped: Number of units per week:
Does the patient currently use recreational drugs?			Please provide details, including which substances and quantity consumed per week.
If the patient currently does not use recreational drugs, have they done so previously?			Please provide details, including which substances, quantity consumed per week, and years of consumption.

Social situation	Please tick	Notes
At home, completely independent		
At home, mostly independent		Please indicate tasks for which the patient requires assistance.
At home, requiring assistance from family or carers.		Please indicate frequency of carers, and tasks for which the patient requires assistance.
Residential home		Please indicate tasks for which the patient requires assistance.
Nursing home		Please indicate tasks for which the patient requires assistance.
Other e.g. sheltered accommodation, warden-controlled premises etc.		Please provide details.

Mobility	Please tick	Notes
Independent		
One stick		
Two sticks		
Frame		
Other walking aid		Please provide details.
Chair/bedbound		
If chair/bedbound, is the patient able to transfer independently between chair and bed?		If no, please indicate level of assistance required by patient for transfer: ☐ Assistance from one person ☐ Assistance from two people ☐ Hoist transfer

Education	Age (years)	Notes
How old was the patient when they left school?		
If the patient went on to higher education, at what age did they leave full time education?		Please indicate highest level of educational attainment achieved by patient (e.g. qualification).

CLINICAL EXAMINATION

Height	CM
Weight	KG
Temperature	^{o}C

Blood Pressure	mmHg
Heart Rate	BPM
MOCA score	

Please see Appendix 1 for MOCA score.

TGUG (see below)	seconds

TGUG = Timed Get Up and Go Test
Please ask participant to rise from a chair, walk 3 metres, walk back to chair, and sit down again. Record the time taken do all of this, in seconds.

	Yes	No
Does the patient have a normal neurological examination?		

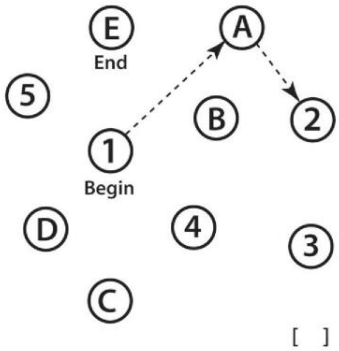
If no, please provide details of any abnormalities below.

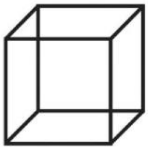
Appendix 1: The Montreal Cognitive Assessment (MOCA)

MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — POINTS





Copy cube

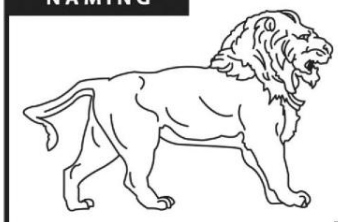
[ ] [ ]

Draw CLOCK (Ten past eleven)
(3 points)

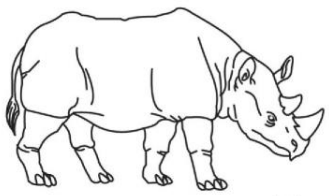
[ ] Contour [ ] Numbers [ ] Hands

__/5

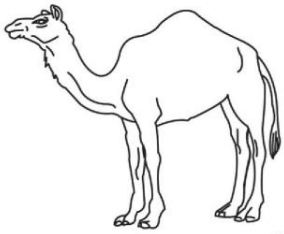
NAMING



[ ]



[ ]



[ ]

__/3

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

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.).

Subject has to repeat them in the forward order [ ] 2 1 8 5 4
Subject has to repeat them in the backward order [ ] 7 4 2

__/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

__/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

__/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ]

__/2

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

__/1

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

__/2

DELAYED RECALL

	FACE	VELVET	CHURCH	DAISY	RED	Points for UNCUED recall only
Has to recall words **WITH NO CUE**	[ ]	[ ]	[ ]	[ ]	[ ]	
Optional Category cue						
Optional Multiple choice cue						

__/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

__/6

© Z.Nasreddine MD www.mocatest.org Normal ≥ 26 / 30

TOTAL __/30
Add 1 point if ≤ 12 yr edu

Administered by: _____

IX. Healthy Volunteer Case Report Form

Biomarkers and Outcomes in CAA (BOCAA)

Case Report Form (CRF)

Please fill in this form for each participant in **BLOCK CAPITALS** and **BLACK INK**.
Please enter the participant's unique study number on each page.

Participant Initials	
Study ID	

Date of CRF completion	DD / MM / YYYY
Name of person completing CRF	
Signature	

PAST MEDICAL HISTORY

Condition	Yes	No	If yes, please provide date(s) of diagnosis.
Hypertension			
Hypercholesterolaemia			
Diabetes Mellitus Type 1			
Diabetes Mellitus Type 2			
Myocardial Infarction			
Cardiac revascularisation (either PCI or CABG)			
Congestive heart failure			
Angina			
Atrial fibrillation			
Peripheral vascular disease			
Dementia or other cognitive impairment			
Seizures			
Migraine with aura			
Migraine without aura			
Transient focal neurological episodes			
Previous ischaemic stroke			
Previous TIA			
Previous ICH			

Please use this space to provide details of any other conditions or procedures. Please include dates where possible.	

Abbreviations:

CABG	Coronary Artery Bypass Grafting
ICH	Intracerebral haemorrhage
PCI	Percutaneous Coronary Intervention
TIA	Transient Ischaemic Attack

CURRENT MEDICATIONS

Drug Name	Dose	Frequency

Any Allergies?

SOCIAL HISTORY			
	Yes	**No**	**If yes, please provide further details below.**
Does the patient currently smoke?			Number of cigarettes per day: If the patient smokes tobacco in another form, e.g. pipes, roll ups, etc., please provide details below (method and quantity per day).
If the patient is currently a non-smoker, have they previously ever smoked?			Year started: Year stopped: Number of cigarettes per day:
Does the patient currently drink alcohol?			Number of units per week:
If the patient currently does not drink alcohol, have they done so previously?			Year started: Year stopped: Number of units per week:
Does the patient currently use recreational drugs?			Please provide details, including which substances and quantity consumed per week.
If the patient currently does not use recreational drugs, have they done so previously?			Please provide details, including which substances, quantity consumed per week, and years of consumption.

Social situation	Please tick	Notes
At home, completely independent		
At home, mostly independent		Please indicate tasks for which the patient requires assistance.
At home, requiring assistance from family or carers.		Please indicate frequency of carers, and tasks for which the patient requires assistance.
Residential home		Please indicate tasks for which the patient requires assistance.
Nursing home		Please indicate tasks for which the patient requires assistance.
Other e.g. sheltered accommodation, warden-controlled premises etc.		Please provide details.

Mobility	Please tick	Notes
Independent		
One stick		
Two sticks		
Frame		
Other walking aid		Please provide details.
Chair/bedbound		
If chair/bedbound, is the patient able to transfer independently between chair and bed?		If no, please indicate level of assistance required by patient for transfer: ☐ Assistance from one person ☐ Assistance from two people ☐ Hoist transfer

Education	Age (years)	Notes
How old was the patient when they left school?		
If the patient went on to higher education, at what age did they leave full time education?		Please indicate highest level of educational attainment achieved by patient (e.g. qualification).

CLINICAL EXAMINATION

Height	CM
Weight	KG
Temperature	^{o}C

Blood Pressure	mmHg
Heart Rate	BPM
MOCA score	

Please see Appendix 1 for MOCA score.

TGUG (see below)	seconds

TGUG = Timed Get Up and Go Test
Please ask participant to rise from a chair, walk 3 metres, walk back to chair, and sit down again. Record the time taken do all of this, in seconds.

	Yes	No
Does the patient have a normal neurological examination?		

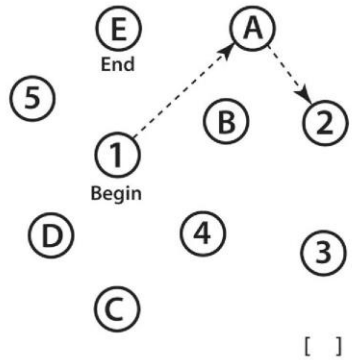
If no, please provide details of any abnormalities below.

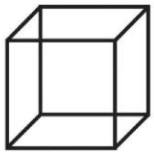
Appendix 1: The Montreal Cognitive Assessment (MOCA)

MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — POINTS

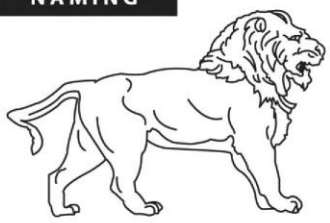
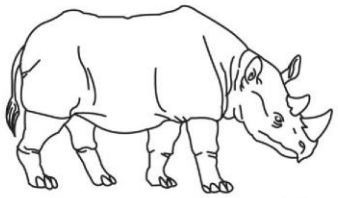
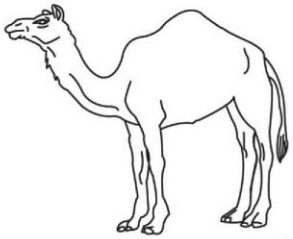




Copy cube [ ] [ ]

Draw CLOCK (Ten past eleven) (3 points) [ ] Contour [ ] Numbers [ ] Hands __/5

NAMING

[ ] [ ] [ ] __/3

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

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

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

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B __/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** __/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ] __/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words) __/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler __/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE	VELVET	CHURCH	DAISY	RED	Points for UNCUED recall only
		[ ]	[ ]	[ ]	[ ]	[ ]	
Optional	Category cue						
	Multiple choice cue						

__/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City __/6

© Z.Nasreddine MD **www.mocatest.org** Normal ≥ 26 / 30

TOTAL __/30
Add 1 point if ≤ 12 yr edu

Administered by: _____

X. Lumbar Puncture Standard Operating Procedure

- All participants in this study will have a lumbar puncture.
- This will be performed at visit 2.
- Visit 2 will take place within 2 weeks of visit 1.

ABBREVIATIONS:
CSF Cerebrospinal fluid
GB Gargi Banerjee, study co-ordinator
LP Lumbar puncture
LWBL Leonard Wolfson Biomarker Lab

EQUIPMENT CHECKLIST PRIOR TO STARTING	
<u>Equipment for performing the lumbar puncture:</u> ☐ Sterile Pack ☐ Cleaning material (e.g. chlorhexidine) ☐ Orange needle ☐ Green needle ☐ 5ML or 10ML syringe ☐ Lignocaine ☐ Spinal needle ☐ Dressing	*Equipment according to LPC preference*
<u>CSF bottles for research tests:</u> ☐ Yellow universal containers x 1	*To be collected by GB for LWBL processing*
☐ Research Consent Form	*This should have been completed on visit 1, and should be in the participant notes.*
☐ LP Consent Form	*Standard NHS Yellow Consent Form*

<u>Procedure:</u>

- Research labels and empty bottles for research samples to be provided by GB on the morning of visit 2
- GB to check blood tests and imaging prior to LP and to inform LPC and LWENC CRF if any contraindications to procedure
- GB to confirm Research Consent Form completed and in participant notes
- GB to complete yellow NHS consent form for procedure and file in participant notes
- GB to perform LP; <u>samples for research only</u>:
    - Yellow universal containers x 1; each has maximum capacity of 25ML
    - The tube should be nearly full (approx. 20ML total)
    - Label both bottles with research labels (provided by GB); no NHS details must be included on these samples
    - GB to contact LWBL and inform them that samples are ready for collection; GB to transport samples to LWBL
    - LWBL MUST RECEIVE SAMPLES BY 4PM AT THE VERY LATEST
- GB to document procedure in participant notes
- Participant to rest for approximately 1 hour, after which they may return home.

XI. Blood Sample Standard Operating Procedure

- All participants in this study will have fasting blood tests.
- This will be performed first thing on Visit 1, after which the participant will be provided breakfast

ABBREVIATIONS:
GB Gargi Banerjee, study co-ordinator
HCP Health care professional who will be taking the blood
LWBL Leonard Wolfson Biomarker Lab

EQUIPMENT CHECKLIST PRIOR TO STARTING	
<u>Equipment for taking blood:</u> ☐ Tourniquet ☐ Cleaning material (e.g. chlorhexidine wipe) ☐ Needle ☐ Vacutainer or syringe ☐ Dressing	*Equipment according to HCP preference*
<u>Blood for NHS tests:</u> ☐ Purple x 1 (EDTA) ☐ Gold x 3 (SST) ☐ Blue x 1 (citrate) ☐ Grey x 1 (fluoride)	*To be sent for NHS blood tests i.e. full blood count, U&E, LFT including total protein, bone profile, ferritin, fasting lipid profile, clotting, and glucose.*
<u>Blood bottles for research tests:</u> ☐ Purple x 3 (EDTA) ☐ Gold x 3 (SST)	*To be collected by GB for LWBL processing.*
☐ Research Consent Form	*This should have been completed at the beginning of visit 1, and be in the participant notes.*

<u>Procedure:</u>

1. Research labels and completed NHS blood test form to be provided by GB on the morning of visit 1
2. HCP to confirm Research Consent Form completed and in participant notes
3. HCP to take blood:
   - NHS tubes to be filled first:
     i. Purple x 1, Gold x 3, Blue x 1, Grey x 1
     ii. Each bottle should be completely filled
     iii. Label each bottles with NHS details i.e. UCLH hospital number; no research details should be included on these samples
     iv. Send in UCLH form for full blood count, U&E, LFT including total protein, bone profile, ferritin, fasting lipid profile, clotting, and glucose (form will be completed by GB prior to procedure)
     v. GB will check results of these tests and contact patient if need be

- Research tubes:
  i. Purple x 3, Gold x 3
  ii. Each bottle should be completely filled
  iii. Label all bottles with research labels (provided by GB); no NHS details must be included on these samples
  iv. HCP to contact GB and inform her that samples are ready for collection
  v. GB to contact LWBL and inform them that samples are ready for collection; GB to transport samples to LWBL
  vi. LWBL MUST RECEIVE SAMPLES BY 4PM AT THE VERY LATEST

4. Participant may then continue with remaining investigations

XII. Patient Follow up Questionnaire

Please complete the questionnaire below, and return to us in the enclosed envelope.

The questionnaire asks your information about your health since the last questionnaire (or your visit). It should only take a few minutes of your time.

If you need help, a carer, friend or relative may fill in this form with you or on your behalf.

Patient Name	
BOCAA study number	
Address	
Is the address we have for you up to date?	**No** ☐ **Yes** ☐ If NO, please enter the new address:

Who completed this form (please circle)? Patient Carer

Friend/relative Other

Please enter today's date: _____ /_____ /_____

1. What is your current living and mobility situation NOW? (please tick Yes or No to all of the following boxes as appropriate)

	Yes	No
Could you live alone without any help from another person? This means being able to bathe, use the toilet, shop, prepare or get meals and manage finances.		
Are you able to do everything that you were doing 6 months ago, even if slower and not as much?		
Are you exactly the same as you were 6 months ago?		
Are you able to walk without help from another person?		
Are you bedridden or needing constant supervision?		

Do you have symptoms unrelated to CAA that could affect your answer to question 1?		

If **YES**, please briefly state your symptoms:

_____

2. We would like to know if you had any further events that may have been a stroke or a mini-stroke since the last questionnaire.

Have you experienced any of the following?	Yes	No
Have you been told by a doctor or other healthcare professional that you have had another stroke in the last 6 months?		
Have you been told by a doctor or other healthcare professional that you have had a TIA, mini-stroke, or transient ischaemic in the last 6 months?		
Have you been told by a doctor or other healthcare professional that you have had another brain haemorrhage in the last 6 months?		
Have you had a sudden painless weakness on one side of your body in the last 6 months?		
Have you had a sudden numbness or a dead feeling on one side of your body in the last 6 months?		
Have you had a sudden painless loss of vision in one eye or both eyes in the last 6 months?		
Have you suddenly lost one half of your vision in the last 6 months?		
Have you suddenly lost the ability to understand what people are saying in the last 6 months?		
Have you suddenly lost the ability to express yourself verbally or in writing in the last 6 months?		
Have you had any sudden vertigo or imbalance in the last 6 months?		
Have you had any loss of coordination of one limb or two limbs in the last 6 months?		
Have you been told by a doctor that you had a heart attack in the last 6 months?		

Have you been told by a doctor that you had angina in the last 6 months?		
Have you had episodes of chest pain in the last 6 months?		
Have you had any paraesthesias (pins and needles, tingling) affecting one side of your body in the last 6 months?		
Have you had any shaking/jerking in one side of your body in the last 6 months?		
Have you had any new problems with vision (for example seeing zig-zags, flashing lights, objects changing their size or shape, or seeing things that were not there) in the last 6 months?		

If you have experienced any of these symptoms did you seek any medical advice?		

If YES, where did you seek advice (please circle)?

Hospital Visit GP visit NHS direct

Name of hospital you visited:

_____

Name of consultant you visited:

_____

THANK YOU for completing this form.

Please put the form in the envelope provided and send it to:

Dr Gargi Banerjee
[redacted]

You can contact the Study Co-ordinator by phoning [redacted] or by emailing [redacted]

XIII. General Practitioner Follow Up Questionnaire

This questionnaire is about one of your patients who agreed to participate in our study. We would be very grateful if you could complete and return it to us in the enclosed envelope (no stamp required).

Patient Name	
BOCAA study number	
Address Date of study recruitment	
Is the address we have for the patient up to date?	**No** ☐ **Yes** ☐ If NO, please enter the patient's new address:

Has the patient had any of the following further events since the study recruitment?					
Ischaemic stroke	☐	TIA	☐	Myocardial infarction	☐
Intracranial haemorrhage	☐	Death	☐	None of these events have occurred	☐

If the patient was admitted to hospital please give the name of the hospital and the date of admission. Please send copies of the discharge summary if possible.
Name of hospital _____ Date of admission _____ /_____ /_____
If the patient has died, please give date of death.
Date of death _____ /_____ /_____

Has the patient received a formal diagnosis of dementia? If so, please indicate subtype.	
☐ Alzheimer's disease	☐ Frontotemporal dementia
☐ Vascular dementia	☐ Other

If other, please specify

_____

Please provide date of diagnosis, if possible _____ /_____ /_____

What is the patient's Modified Rankin Scale score? Please circle.	
0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties and activities
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6	Dead

THANK YOU for completing this form.

Please put the form in the envelope provided and send it to:

Dr Gargi Banerjee
[redacted]