Applied Clinical Neuroimaging in Cerebral Amyloid Angiopathy and Spontaneous Intracerebral Haemorrhage

by

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DECLARATIONS

I, Andreas Charidimou confirm that the work presented in this thesis entitled "Applied Clinical Neuroimaging in Cerebral Amyloid Angiopathy and Spontaneous Intracerebral Haemorrhage" is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

I collected, reviewed, cleaned and analysed (including imaging and statistical analysis) the data included in this thesis. I personally performed the searches, the collection of papers and the data extraction for the systematic reviews incorporated in this thesis. Dr Simone Gregoire significantly contributed in collecting data for the multicentre cohort of UCLH, Belgium and Cambridge (UK). Dr Young T. Hong collected the PET and MRI data included in Chapter 7 from the Wolfson Brain Imaging Centre, Department of Clinical Neurosciences, University of Cambridge, UK. Dr Sergi Martinez-Ramirez, Dr Anand Viswanathan, Prof Steve Greenberg and other members of the Hemorrhagic Stroke Research Program in Massachusetts General Hospital Stroke Research Center, Harvard Medical School, Boston, MA, USA, contributed in setting up the cerebral amyloid angiopathy pathology cohort which appears in Chapter 10.

I was supported in the statistical approaches and analyses by Dr Zoe Fox, the statistician associated with the UCL Institute of Neurology. Finally, this thesis was drafted in its entirety by myself, but with critical revisions by my principal supervisor, Dr David Werring and my subsidiary supervisor, Dr Hans R. Jäger. Sections of this thesis have been published in peer-reviewed scientific journals as stated below. Finally, Prof. Jean-Claude Baron provided critical revisions for the study presented in Chapter 7.

Signature:

ABSTRACT

Sporadic cerebral amyloid angiopathy is a common small vessel disease that preferentially involves small cortical and leptomeningeal arteries due to progressive amyloid-β deposition in their walls. Cerebral amyloid angiopathy occurs frequently in elderly people, and is a common and important cause of symptomatic lobar intracerebral haemorrhage and cognitive impairment. There is currently a growing interest in cerebral amyloid angiopathy, at least partly thanks neuroimaging, which now allows an unprecedented ability to investigate the disease dynamics *in vivo* using MRI to reveal complex patterns of cerebral bleeding and ischaemia. The detection of CAA during life is becoming an increasingly important challenge, since approaches of prevention or treatment (disease-modification) are now emerging as realistic possibilities. Determining the most promising treatments requires development of reliable biomarkers, the goal of my research. The main objective of this PhD thesis is to provide new insights into potential clinical and applied clinical neuroimaging biomarkers in patients with cerebral amyloid angiopathy. This is accomplished by a portfolio of research studies investigating: (a) the clinical and radiological spectrum of transient focal neurological episodes as a potential clinical clue for cerebral amyloid angiopathy; (b) cortical superficial siderosis, a distinct pattern on bleeding in the brain, as both a diagnostic and a prognostic marker of cerebral amyloid angiopathy; (c) MRI-visible perivascular spaces topography, as a new marker of small vessel disease and cerebral amyloid angiopathy; (d) potential pathological, neuroimaging and genetic differences in patients with pathology-proven CAA with and without intracerebral haemorrhage and presents evidence for different disease phenotypes; (e) the evidence whether the presence and burden of cerebral microbleeds on MRI scans is associated with an increased risk of recurrent spontaneous ICH, and if this risk is different according to MRI-defined microangiopathy subtype, in a meta-analysis.

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For my family, for the love and opportunities you have given me,

and, for Maria,

who have been there for me through everything,

with love

Chapter 1 General Introduction

Sporadic cerebral small vessel disease: key definitions and current concepts

Many of the modern advances and effective interventions in cerebrovascular disorders currently target only disease of large arteries. Until recently, small cerebral arteries have received little attention and clinicians have much less to offer for the prevention and treatment of small vessel disease.[1] This is partly because small vessels are technically inaccessible, hard to study directly and hence the underlying mechanisms of small vessel disease remain relatively poorly understood.[2] Yet, these diseases are considered to be among the most prevalent known neurologic processes and contribute substantially to stroke, cognitive impairment or other disabilities commonly seen in elderly persons (e.g. depression, motor and gait disturbances, urinary symptoms, functional impairment etc.).[2-4] In addition, small vessel disease can increase mortality.[5, 6]

Although the term sporadic cerebral small vessel disease is used with various meanings in different contexts (e.g. clinical, neuroimaging, pathological etc.), in its most basic form it encompasses a group of age-related neuropathological processes affecting the small arteries, arterioles, capillaries, and rarely venules in the brain.[1-3] Before defining these pathological processes, it is important to define what a small vessel is and specifically, "how small a small vessel is". Interestingly, a survey performed among leading neuropathological centres, revealed that the definition of a small vessel is not consistent: less than 50% of the participants agreed on a size limit of less than 500 μm in transverse diameter or all vessels located deeper than in the cortex.[7] Others have arbitrarily suggested a transverse diameter of ≤300 μm, predominantly referring to arterioles – likely illustrating that pathological processes of the arteriolar tree are more well know that those of other small vascular components (e.g. capillaries).[2] The current definition of small vessels is more inclusive, referring to all vascular structures (ranging from around 5μm up to 2mm), small arteries, arterioles, capillaries, venules and small veins located in the brain parenchyma (i.e. intraparenchymal) or in the subarachnoid space (i.e. leptomeningeal).[2] These small vessels can either: (a) penetrate the brain cortex superficially, supplying the gray matter with short branches of three lengths (reaching cortical layer III, V and the gray–white matter junction), and the subcortical white matter with longer branches; or (b) stem from arterial perforators deeper at the base of the brain and supplying the basal ganglia, thalami and brainstem structures.[2] Specific small vessel pathologies can differentially affect these two systems as well as different range of vessels within each system (Figure 1.1).

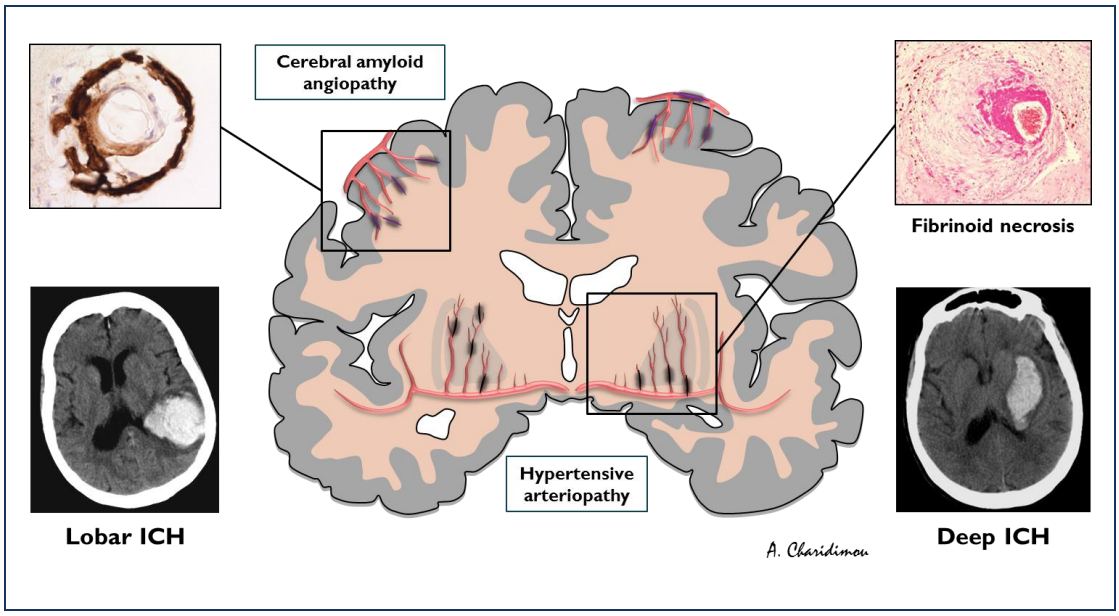

Figure 1.1 The topography of sporadic cerebral small vessel disease. The small vessels of the brain can mainly be affected by two types of pathological process: (1) hypertensive arteriopathy – which typically affects small deep arterial perforators; or (2) cerebral amyloid angiopathy (CAA) – which preferentially affects the small arteries and arterioles of the cerebral cortex and gray–white-matter junction by the deposition of amyloid-β in the vessel walls.

The overall rationale behind this definition of small vessels *versus* large vessels is twofold. First, this separation highlights that the pathological processes affecting small vessels in the brain are different from those that affect large vessels. It needs to be recognised that often this assumption is only partially true as small and large vessel disease processes can to a certain extent overlap and often co-exist in elderly individuals (e.g. hypertension-related atherosclerosis and arteriolosclerosis). In addition, the two subtypes of cerebrovascular disease can be driven by very similar risk factors, not the least of which is simply ageing. It is also important to keep in mind that large and small vessel systems form a continuum and therefore there is a cross-talk between aging large arteries and microvascular brain injury. For example, progressive age-associated increase in large artery stiffness exposes small vessels to higher pulse pressure which in turn can lead to progressive white matter rarefaction and cognitive impairment.[8] Although subdividing cerebrovascular disease into micro-vascular (i.e. small vessel) macro-vascular (i.e. large vessel) components represent a gross oversimplification, this distinction serves the concept that not only the putative pathophysiology, but also the clinical consequences and the injury each group of diseases cause on the brain parenchyma are different. Approximately one third of symptomatic strokes are caused by diseases of small perforating arteries and arterioles of the brain, including lacunar stroke syndromes[9] and most cases of spontaneous intracerebral haemorrhage (ICH) - perhaps the most severe and lethal type of stroke.[1] However, beyond its role in clinically overt acute stroke syndromes, small vessel disease

causes widespread microvascular damage (seen on neuroimaging or at autopsy) which is not symptomatic itself but has important cumulative effects on cognition. Hence, cerebral small vessel disease is one of the most important contributors to cognitive impairment in the elderly.[10, 11] As people live longer, the burden of these diseases will rapidly grow over the coming years becoming an increasing healthcare challenge facing all societies worldwide.[12]

There are two main sporadic forms of small vessel disease (Figure 1.1). The first one is sporadic cerebral amyloid angiopathy (CAA) (extensively covered in the next section of the Introduction), a chronic degenerative disease characterised with progressive deposition of amyloid-β in the media and adventitia of small arteries, arterioles and capillaries in the cerebral cortex, overlying leptomininges and grey–white matter junction.[10, 11] In contrast to CAA which is relatively easy to define, the remaining sporadic small vessel disease is more difficult to define and name. To this end, the term "hypertensive arteriopathy" is widely used to describe a non-amyloid process often related to advanced age (but not clearly age-driven), hypertension, diabetes mellitus and other common vascular risk factors, typically affecting the small perforating end-arteries of the deep grey nuclei and deep white matter.[2] It is characterised pathologically by lipohyalinosis, arteriolosclerosis or fibrinoid necrosis. This very common sporadic form of small vessel disease has been variously known as arteriolosclerosis, age-related or vascular risk-factor-related small vessel disease, or degenerative microangiopathy in the literature.[2, 13, 14] The term "hypertensive arteriopathy" is not ideal (or even misleading) as it probably groups together a variety of sporadic small vessel disease pathologies not accounted by sporadic CAA, but not necessarily related specifically to hypertension.[15] In other words, hypertension might not be a specific cause of these small vessel changes collectively called "hypertensive arteriopathy" (at least not in all cases), but can to a certain extent influence their evolution. Furthermore, there is not a consensus on the microscopic small vessel lesions best described under the term "hypertensive arteriopathy", meaning that its severity might be difficult to evaluate in any given case. From a histopathologic perspective, hypertensive arteriopathy is mainly characterised by vessel wall thickening, narrowing of the lumen, loss of smooth muscle cells from the tunica media and deposits of an amorphous fibro-hyaline material. Other possible pathological features can include distal manifestations of atherosclerosis (microatheroma) and the presence of microaneurysms (i.e. elongated and dilated vessels).[2] However, many researchers tend to subdivide hypertensive arteriopathy based on the most pronounced structural histopathological features found, e.g. atherosclerosis, arteriolosclerosis, lipohyalinosis, fibrinoid necrosis (the proposed acute from of lipohyalinosis), microaneurysms etc.[2] These subtypes tend to predominantly affect different small vessel

sizes and can exist separately or in various combinations in any given case. Of these histopathological features, perhaps the one most strongly associated with hypertension-related injury is fibrinoid necrosis, which is much more common in hypertensive patients' brains than in those without hypertension,[16-18] as well as in arterioles adjacent to deep ICH.[17-21] Complicating matters further, the more effective treatments for hypertension in recent years is likely to have modified the specific pathological features, natural history and disease spectrum of hypertensive arteriopathy.[15] Despite the limitations in definitions and given the lack of an alternative widely accepted term, for simplicity and consistency, the term "hypertensive arteriopathy" has been adopted in this thesis as one of convenience to avoid unnecessarily long and complex terms being repeated. Whatever term one prefers for this sporadic small vessel disease type, the microvascular changes associated with hypertensive arteriopathy presumably lead to both occlusion (including reduced vessel compliance and impaired vasoreactivity) and haemorrhage in the brain territories of the affected deep perforating arteries. Unsurprisingly, hypertensive arteriopathy has historically been associated with two types of cerebrovascular disease: lacunar infarcts[22] and intracerebral haemorrhage.[23]

Since small vessels (and hence the structural alterations of small vessel disease) cannot be easily visualised *in vivo* with the current neuroimaging techniques used in clinical practice, the brain parenchymal magnetic resonance imaging (MRI) lesions which they are thought to cause have been adopted as markers of small vessel damage.[2] As a result, the term cerebral small vessel disease is frequently used indiscriminately to describe both the underlying small vessel pathologies and the neuroimaging correlates of their consequences on the brain parenchyma.[2] Of note, these consequences are heterogeneous in nature including both ischaemic and haemorrhagic manifestations.[2, 24] However, historically the term small vessel disease has been often (and still is) used misleadingly to describe only the ischaemic consequences on imaging.[3] Despite this, sporadic small vessel disease is the leading cause of ICH, which in fact parallels the topography of the underlying microvascular pathology, so that spontaneous deep ICH (in the basal ganglia, thalami etc.) is predominantly caused by hypertensive arteriopathy, whereas lobar (cortical-subcortical) ICH is frequently caused by CAA. Lacunes and white matter hyperintensities (leukoaraiosis) are well known imaging markers of cerebral small vessel disease that have been extensively studied with MRI since the early 90s.[25, 26] Advances in neuroimaging, now allows an unprecedented ability to investigate more complex dynamics (both haemorrhagic and non-haemorrhagic) of small vessel disease *in vivo*: new imaging manifestations of small vessel disease include cerebral microbleeds,[27] cortical superficial siderosis (cSS) and convexity subarachnoid haemorrhage,[28] cerebral microinfarcts[29] and perivascular spaces.[30]

A further concept to bear in mind when approaching neuroimaging markers of small vessel disease (and hence markers of pathologic consequences on the brain parenchyma), is that their pathogenic interpretation is not uniform, and given marker may be found in different types of small vessel disease.[24] For example, in view of different topographical distribution of hypertensive arteriopathy and CAA, it is hypothesized that cerebral microbleeds have a preferential location depending on the underlying small vessel pathology: hypertensive arteriopathy is commonly associated with cerebral microbleeds in deep brain regions (e.g. basal ganglia, thalamus and brainstem), whereas CAA is characterised by cerebral microbleeds in a lobar distribution (cortical-subcortical).[27] Hence, each marker or lesion on neuroimaging should not be taken in isolation.

These advances in neuroimaging were recently illustrated in an international working group position paper from the Centres of Excellence in Neurodegeneration under the acronym STandards for ReportIng Vascular changes on nEuroimaging (STRIVE v1).[31] This consensus paper presents a unified approach to small vessel disease at present as a neuroimaging-defined concept, which is changing rapidly paralleling the continued advances in neuroimaging techniques. Table 1.1 provides a glossary and definitions of the most commonly used research terms in the field of small vessel disease (a defined in the STRIVE consensus paper), including MRI modalities.

In the next section of the introduction a closer look at CAA is provided, since this type of small vessel disease is the main focus of the research presented in this PhD thesis. Given that hypertension is theoretically treatable, whereas CAA is currently not, and considering that CAA is strongly associated with brain aging and is also directly linked to Alzheimer's disease, is not surprising that CAA is becoming the most common sporadic small vessel disease, at least in Western populations.[32] This is partly illustrated by the changing profiles of spontaneous ICH in recent epidemiological studies, showing an increase in ICH incidence among patients aged 75 years or older, especially in relation to antithrombotic use.[33, 34] Also, while the incidence of deep (probably hypertensive arteriopathy-related) ICH has fallen, the proportion of non-hypertensive lobar bleeds in those aged 75 years or over increased. It is likely that CAA is implicated in the majority of these lobar haemorrhages and might also account for the increased incidence of anticoagulation-associated ICH.[28]

Table 1.1 Research definitions of commonly used terms in the field of cerebral small vessel disease[31] as well as basic structural MRI techniques.[35]

Proposed definitions for neuroimaging features of small vessel disease

- **Recent small subcortical infarct:** Neuroimaging evidence of recent infarction in the territory of one perforating arteriole, with imaging features or clinical symptoms consistent with a lesion occurring in the previous few weeks.
- **Lacune of presumed vascular origin:** A round or ovoid, subcortical, fluid-filled cavity (signal similar to CSF) of between 3 mm and about 15 mm in diameter, consistent with a previous acute small subcortical infarct or haemorrhage in the territory of one perforating arteriole.
- **White matter hyperintensity of presumed vascular origin:** Signal abnormality of variable size in the white matter that shows the following characteristics: hyperintensity on T2-weighted images such as fluid-attenuated inversion recovery, without cavitation (signal different from CSF). Lesions in the subcortical grey matter or brainstem are not included in this category unless explicitly stated. If deep grey matter and brainstem hyperintensities are also included, the collective term should be subcortical hyperintensities.
- **Perivascular spaces:** Fluid-filled spaces that follow the typical course of a vessel as it goes through grey or white matter. The spaces have signal intensity similar to CSF on all sequences. Because they follow the course of penetrating vessels, they appear linear when imaged parallel to the course of the vessel, and round or ovoid, with a diameter generally smaller than 3 mm, when imaged perpendicular to the course of the vessel.
- **Cerebral microbleed:** Small (generally 2–5 mm in diameter, but sometimes up to 10 mm) areas of signal void with associated blooming seen on T2*-weighted MRI or other sequences that are sensitive to susceptibility effects.
- **Brain atrophy:** A lower brain volume that is not related to a specific macroscopic focal injury such as trauma or infarction. Thus, infarction is not included in this measure unless explicitly stated.

The basic structural neuroimaging toolbox

- **Spin-echo sequences:** conventional MRI pulse sequences in which both a 90° excitation pulse and a 180° refocusing pulse are used to generate an MR signal. The 180° refocusing pulse has the effect of reducing the susceptibility to magnetic field inhomogeneities, and thus leading to a reduced sensitivity to detect microbleeds, for example.
- **Fast spin-echo sequences:** a type of spin-echo technique that uses a series of multiple rapid 180° refocusing pulses and multiple echoes (echo train) and is in standard use for T2-weighted MRI because of the shortened scan duration.
- **T2*-weighted gradient-recalled echo sequence:** an MRI pulse sequence that lacks a 180° refocusing (rephasing) pulse and is therefore more sensitive to magnetic susceptibility effects than are spin-echo or fast spin-echo techniques.
- **Magnetic susceptibility:** a natural property of tissues, which reflects the magnetic response of a substance to an external magnetic field. The susceptibility differences between substances lead to local magnetic field inhomogeneities, resulting in faster T2* relaxation which leads to signal loss on MR sequences sensitive to T2* effects. The haemosiderin deposits within cerebral microbleeds are paramagnetic, and hence have a strong susceptibility effect, which increases local dephasing of spins allowing them to be more easily detected using sensitive MRI techniques.
- **Susceptibility effect:** refers to the fast decay of the local MRI signal caused by the internal magnetisation of tissue that generates local inhomogeneity in the magnetic field.
- **Susceptibility-weighted imaging:** a term applied to MRI data acquisition techniques that employ a high resolution 3D gradient-recalled echo sequence with a long echo time and generate additional contrast for magnetic susceptibility by use of phase imaging combined with magnitude data in an image post-processing step.

Sporadic cerebral amyloid angiopathy: pathophysiology and clinical spectrum

Sporadic cerebral amyloid angiopathy (CAA) is a common small vessel disease of the brain, characterised by the progressive deposition of amyloid–β protein in the walls of small-to-medium sized arteries (up to about 2mm in diameter[36]), arterioles and capillaries in the cerebral cortex and overlying leptomininges.[37, 38] CAA can also affect cerebellar vessels, but only rarely those in the brainstem or basal ganglia. Although known to pathologists for over a century,[39, 40] CAA was not linked to clinical disease until as late as the 1960's, when it was suggested to be a rare cause of intracerebral haemorrhage (ICH).[41-43] In recent years, CAA has been "rediscovered" as a common and important cause of spontaneous ICH, which remains the most devastating form of stroke, with a fatality rate approaching 50% in contrast to improved outcomes from ischaemic stroke.[44, 45] An increased understanding of CAA thus holds promise for improved prevention and treatment of ICH.

The growing interest in CAA is at least partly thanks to two fields of research, which have been important in defining the expanding clinical-radiological phenotype and the underlying pathophysiology of the disease: (1) neuroimaging, which now allows an unprecedented ability to investigate CAA dynamics *in vivo* using MRI to reveal complex patterns of cerebral bleeding (including lobar microbleeds[27]) and ischaemia, and an increasing repertoire of specific amyloid-binding ligands;[38, 46-50] and (2) transgenic mouse studies, which have allowed the experimental alteration of amyloid peptide expression and molecular structure, providing significant mechanistic insights. Despite these advances, CAA remains under-recognized by neurologists and stroke physicians, making a fresh look especially timely (see box for search strategy).

The entity of CAA encompasses a number of highly diverse sporadic and genetic disorders that share the same pathological hallmark of amyloid-β fibril deposition in small leptomeningeal and cortical vessels. Here, we focus only on sporadic CAA.

Search strategy and selection criteria
References were identified through PubMed with the search terms: "cerebral amyloid angiopathy"; "microbleed(s) or microh(a)emorrhage(s) and cerebral amyloid angiopathy"; "intracerebral h(a)emorrhage"; and "vascular cognitive impairment" between January 1970 and August 2014. The references from identified articles and the authors' own files were also searched for relevant publications. Only papers published in English were reviewed. The final reference list was chosen on the basis of relevance to the topics covered in this thesis.

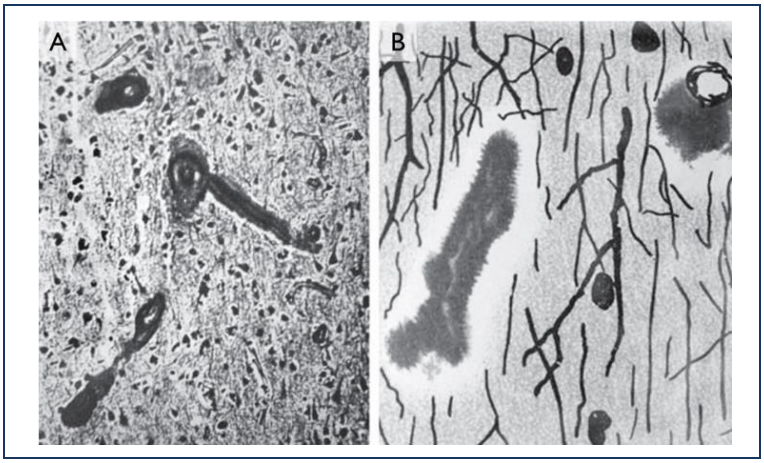

Figure 1.2 The first published photograph of cerebral amyloid angiopathy (left panel), accompanied by the author's illustration (right panel), from Fischer, 1910. Note the "fur-like" staining of the walls of the 3 small arteries.

1.1 Historical perspectives on CAA: from a rare pathological curiosity to a common and devastating disease

In 1907, Alois Alzheimer described the neuropathological findings in a 51- year-old woman with progressive dementia,[51] observing scattered foci, within the cerebral cortex, *"of a peculiar substance which can be recognized without stain and is, in fact, very resistant to staining."* In 1927, Divry discovered that these deposits were amyloid,[52] now recognized to be a fibrillar protein with a powerful tendency to aggregate, and the main component of "senile plaques" that define Alzheimer's disease. The CAA story began around the same time, in 1909, at the Psychiatry Clinic at the University of Freiburg, Germany, with Gustav Oppenheim's first histopathological report of foci of focal necrosis ("drusige Nekrosen") in the brain next to abnormal capillary walls thickened by a translucent ("hyaline") substance.[39]

Soon afterwards, in 1910, Oskar Fischer confirmed occasional "fur-like" staining of small arteries in Alzheimer's disease, and published the first known photograph of CAA (Figure 1.2). Building on these observations, the first systematic study of CAA, was published in 1938 by Scholz,[40] who described a proteinaceous material ("Drusen") in intracortical arteries, capillaries and the immediately adjacent brain tissue, that he termed "drusige Entartung der Arterien und Kapillaren".[40] Other authors coined the term "angiopathie dyshorique" (dyshoric angiopathy)[53, 54] (from the Greek word *horos*=border) implying that blood-derived amyloid might enter the brain (crossing the "blood-brain barrier") leading to amyloid deposition around small vessels.[54]

Figure 1.3 The entrance of the University Psychiatric Clinic in Bel-Air at Geneve, where the University of Geneva brain collection was founded at the beginning of the 20th century. The panel on the right shows the brain of one of Stefanos Pantelakis original brains included in his study on congophilic angiopathy,[55] along with the patients' cognitive evaluation of executive skills (lower panel).

However, in 1954, Stefanos Pantelakis, in the University Psychiatric Clinic in Bel-Air, Switzerland, demonstrated CAA was limited to the vascular wall, without adjacent parenchymal involvement (Figure 1.3), raising the alternative hypothesis that the amyloid may originate in the brain rather than the blood,[55] consistent with modern concepts of amyloid pathophysiology. In his landmark paper, Pantelakis also described what are still considered the pathological hallmarks of CAA: (a) preferential involvement of small arterioles and capillaries of the leptomeninges, cerebral cortex, and cerebellar cortex; (b) a topographical distribution favouring posterior lobar brain regions (especially the occipital lobes); (c) the lack of involvement of white matter small vessels; (d) the association with increased age and dementia; (e) the lack of association with hypertension and arteriosclerosis (the other main small vessel disease, but which preferentially affects the basal ganglia and brainstem); and (f) the lack of any link with amyloidosis of other organs.[55]

Since these early descriptions, understanding of the pathophysiological mechanisms in CAA has increased substantially. As it will become evident throughout this review, similar to the modern concept of cerebral small vessel disease, the term CAA now encompasses not only a specific cerebrovascular pathological trait and disorder, but also a clinical

syndrome (or syndromes) and brain parenchymal lesions seen on neuroimaging (including a set of imaging criteria).

1.2 Epidemiology and risk factors

Pathologically-defined CAA is common in the elderly.[56-59] Population-based autopsy studies indicate a CAA prevalence of 20-40% in non-demented, and 50-60% in demented elderly populations.[58, 60-63] Furthermore, CAA pathology may be severe in older individuals: in the Honolulu-Asia aging autopsy study (HAAS), severe CAA was found in 43% of demented and 24% of non-demented elderly individuals (mean age at death: 85 years).[62] In Alzheimer's disease, CAA is almost invariable, being found at autopsy in more than 90% of cases.[56, 64] However, most of these patients have mild CAA; severe CAA is found in about 25% of Alzheimer's disease brains.[65]

Advancing age is the strongest known clinical risk factor for developing CAA.[37] In a community-based sample of 100 individuals, the prevalence of cortical vascular amyloid-β deposition progressively increased from the 7th to the 9th decades,[66] a pattern also observed in 784 consecutive autopsies, corrected for over-representation of Alzheimer's disease.[67] Moreover, patients with CAA-related ICH (suggesting advanced disease) in large autopsy series, were all older than 60 years (and most over age 70).[42, 68, 69] CAA is seldom reported before the sixth decade of life; occasional patients presenting in their 50s have been described.[70]

By contrast with hypertensive arteriopathy-the other main form of small vessel disease and cause of ICH,[2] the risk of CAA is not accounted for by conventional cardiovascular risk factors other than age.[37] Hypertension is not considered a risk factor for developing CAA, but may increase the risk of CAA-related ICH. Vinters[37] – in a clinicopathological series of 107 pathologically-proven CAA cases – found the prevalence of hypertension to be around 32%, similar to community-dwelling elderly populations,[71] while another pathological study reported that CAA patients with ICH were more frequently hypertensive (50%) than those without ICH (23%), suggesting that hypertension may contribute to CAA-related cerebral bleeding. [72] In a recent multicentre cohort of patients with spontaneous ICH, we found that the prevalence of hypertension in CAA-related ICH was 62% - significantly less than in non-CAA-related ICH (85%).[73] Whether hypertension in association with CAA confers a greater risk for ICH, compared to CAA alone is an important clinical question.[74-76]

Apolipoprotein E (APOE) genotype is the only known genetic risk factor for sporadic CAA.[77] APOE is a protein with crucial roles in lipoprotein complexes, which regulate lipid

metabolism by binding to cell-surface APOE receptors and proteins associated with lipid transfer and lipolysis.[77] There are three major polymorphisms in the APOE gene, namely ε4, ε2 and ε3, resulting in a single amino-acid change[78] which dramatically alters the functional properties of APOE isoforms.[79] These alleles have a strong dose-dependent effect on the risk of developing CAA and its clinical severity. Thus, APOE ε4 in both post-mortem and clinical series increases the risk of sporadic CAA-related lobar ICH; moreover, the number of ε4 alleles relate to clinical severity.[77, 80-82] Individuals carrying the APOE ε2 allele also have an increased risk of CAA-related lobar ICH.[82, 83] Both of these risk alleles are also associated with a younger age of first ICH,[84] greater likelihood of haematoma expansion, poorer clinical outcome,[85, 86] and a higher risk of recurrence.[87] Furthermore, the two allelic variants interact: patients with both APOE ε2 and ε4 alleles have the earliest disease onset and highest risk of early ICH recurrence.[87, 88] The ε2 and ε4 alleles might promote CAA-related haemorrhage through distinct mechanisms: ε4 by promoting amyloid-β deposition; and ε2 by inducing structural changes in amyloid-laden vessels, making them prone to rupture.[85, 86, 88-90] Other as yet unidentified genetic polymorphisms relating to amyloid metabolic pathways may also play a role in sporadic CAA, (for example presenilin-1, neprilysin and transforming growth factor beta-1),[91-93] and are a topic of ongoing investigation.

1.3 Neuropathology

1.3.1 Morphological characteristics, natural history and severity grading

CAA primarily involves neocortical and leptomeningeal arterioles, to a lesser extent capillaries and, very rarely, venules.[38] In contrast to amyloid plaques found in Alzheimer's disease - which are predominantly composed of the 42 amino-acid residue fragment (amyloid-β_{42}) - the vascular amyloid in CAA is mostly composed of the more soluble, 40 amino-acid fragment (amyloid-β_{40}), suggesting different pathophysiological mechanisms for pathological deposition (see below).[94-97] Cerebral vessels with moderate to severe CAA show an acellular wall thickening with a strongly eosinophilic smudgy appearance on haematoxylin-eosin stained sections.[98] Congo-red staining, under polarized light, reveals amyloid deposits as "apple-green" birefringence (hence the term *congophilic angiopathy*)[37, 99] although immunological stains for amyloid-β are highly specific and now widely used. The development of CAA is progressive, with amyloid-β first appearing in the abluminal aspect

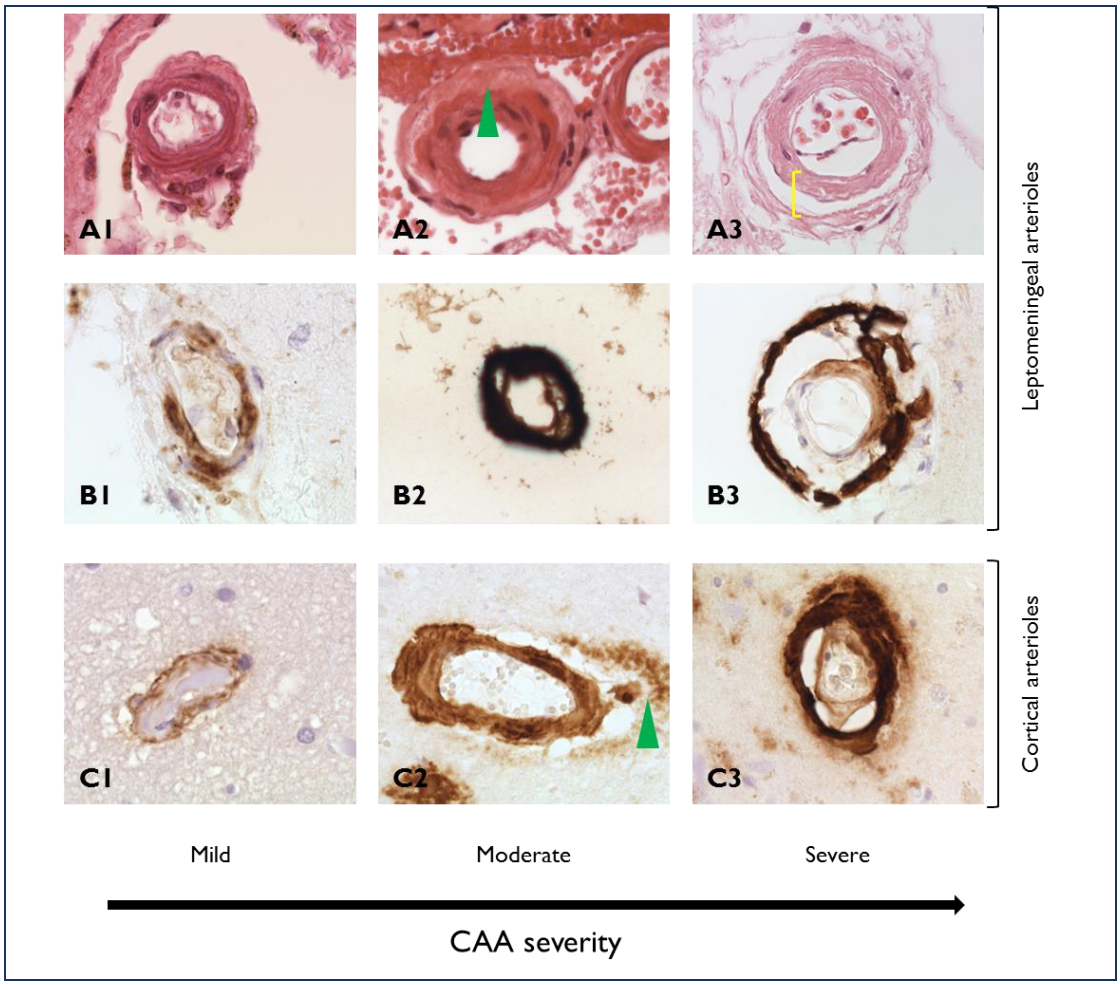

Figure 1.4 Histopathological features of cerebral amyloid angiopathy (CAA) (A1-A3) Morphological changes of the vessel walls of leptomeningeal arterioles as revealed by haematoxylin & eosin staining (H&E) and immunohistochemical detection of amyloid-β. In mild and moderate CAA only minimal structural changes can be detected: in A2 the arrowhead points to amyloid deposition in the vessel wall. However, in advanced CAA, there are significant structural alterations, the most extreme of which is vessel-within-vessel appearance, sometimes termed "double-barrelling" (detachment and delamination of the outer part of the tunica media; bracket in A3). (B1-B3) A similar pathologic range of CAA-related changes in leptomeningeal arterioles using immunohistochemical detection of amyloid-β. In mild CAA (B1) there is a patchy deposition amyloid in the vessels wall. Moderate CAA shows more dense amyloid deposition which spans the entire vessel wall (B2), while severe CAA, shows double-balled vessels and endothelial involvement (B3). (C1-C3) Pathological findings of CAA in cortical arterioles. C2 shows moderate CAA with pan-mural deposition of amyloid-β along with amyloid-β deposition in the surrounding brain parenchyma (arrowhead). In C3 a double-barrel vessels can be seen, although this is less common compared to leptomeningeal vessels.

of the tunica media, surrounding smooth muscle cells, and in the adventitia (Figure 1.4).[37] At the initial stage the vessel wall structure is intact, but as the disease progresses, there is pan-mural amyloid accumulation, and loss of smooth muscle cells.[38] In severe CAA, detachment and delamination of the outer part of the tunica media result in so-called "double barrel" appearance (Figure 1.4);[38] fibrinoid necrosis and microaneurysm formation also occur in advanced disease. There may also be microbleeding with perivascular

deposition of erythrocytes and blood-breakdown products.[98] Endothelial cells are usually preserved even in vessels severely affected by CAA.[100] Occasionally amyloid-β is deposited in the surrounding brain parenchyma immediately adjacent to an affected vessel (sometimes called "dyshoric CAA").

CAA is also associated with cerebral ischaemic damage,[56, 65, 101, 102] including cortical microinfarcts,[103] and white matter pathology (demyelination and gliosis).[43, 56, 96] Microinfarcts are predominantly lobar (cortical-subcortical), usually in patients with severe CAA. One possible mechanism for these ischaemic lesions is occlusion or reduced perfusion in amyloid-laden cortical vessels affected by CAA.

The changes described above provide the basis of neuropathological scoring systems for CAA [72, 101, 104] each with strengths and limitations.[105] No standardized consensus neuropathological criteria for rating CAA are available,[106] but are desirable to allow comparison of CAA pathological studies between centres. A more detailed discussion of CAA severity grading can be found in a recent review by Attems and colleagues.[38]

1.3.2 Pathological subtypes of sporadic CAA

At least two distinct pathological subtypes of CAA have been described: CAA-type 1, characterised by amyloid-β in cortical capillaries (with or without involvement of other vessels)[38]; and CAA-type 2, where amyloid-β deposits are restricted to leptomeningeal and cortical arteries, arterioles and, rarely, veins.[107] Amyloid-β deposition in the wall of capillaries (capillary CAA, also used to be termed "dyshoric angiopathy") may cause luminal obstruction in the most severe stages.[36] The APOE ε4 allele is most strongly associated with CAA-type 1, while APOE ε2 is more associated with CAA-type 2.[107] CAA-type 1 appears to be more closely associated with parenchymal amyloid deposition in Alzheimer's disease.[108]

1.4 Topographical distribution

Sporadic CAA favours posterior cortical regions; the occipital lobe is most frequently affected, followed by the frontal, temporal and parietal lobes.[37, 38] The occipital lobe is also most severely affected.[109, 110] The cerebellum can be affected in advanced stages, while the basal ganglia, thalami, white matter and brainstem are typically spared.[105] The distribution of CAA pathology shows a characteristic patchy pattern,[37] so that foci of vessels severely

affected by CAA may be adjacent to others with mild or absent amyloid-β deposition.[37, 38] The practical consequence of this is that cerebral biopsy may miss patchy CAA pathology.

1.5 Pathophysiological pathways

1.5.1 Amyloid-β production, clearance and accumulation

Amyloid-β is generated by sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretases. Mutations in the gene encoding the APP account for some rare (usually autosomal dominant) forms of CAA, including CAA-Dutch type.[111] Familial non-amyloid-β forms of CAA include familial British dementia[112, 113], familial Danish dementia[114] and Icelandic cystatin C mutation.[115] In general, hereditary forms of CAA have an earlier onset and more severe clinical manifestations than sporadic CAA.[98, 116] Although exceptionally rare, familial CAAs have provided significant insights on how mutations in the coding region of the APP contribute to CAA pathogenesis: for example, the Iowa, Dutch, Italian, and Arctic mutations render amyloid-β highly toxic to vessel wall components,[117-119] and more resistant to proteolytic degradation[120] or clearance from the brain.[121]

Factors that initiate or promote amyloid-β peptide deposition in the much more common sporadic CAA are not as well understood. Nevertheless, transgenic mouse models of cerebral amyloid deposition[38] have provided the following insights (Figure 1.5): (a) the major source of human amyloid-β is neuronal;[122, 123] (b) an increased ratio of amyloid-β_{40} : amyloid-β_{42} in the brain results in a shift from brain parenchyma to the vasculature (perhaps by increasing the solubility of amyloid-β and thus its diffusion into the vessel wall);[124] and (c) vascular amyloid-β deposition largely results from impaired clearance of amyloid-β (rather than overproduction), especially along perivascular drainage pathways.[38, 125, 126] Impairment of perivascular drainage pathways has emerged as a key mechanism in sporadic CAA:[38, 127, 128] these efflux channels may be conceptualized as a cerebral "lymphatic system", allowing interstitial fluid and solutes to drain out of the brain along basement membranes in the capillary walls, and between smooth muscle cells in the tunica media of small arteries (in the opposite direction to arterial blood flow) (Figure 1.5).[129] This transport system is thought to be driven by pulsations of the blood vessel wall.[129, 130] As this and other clearance mechanisms fail in the ageing brain, or under other pathological conditions, amyloid-β is increasingly trapped and deposited in the walls of small arteries (Figure 1.5).[129]

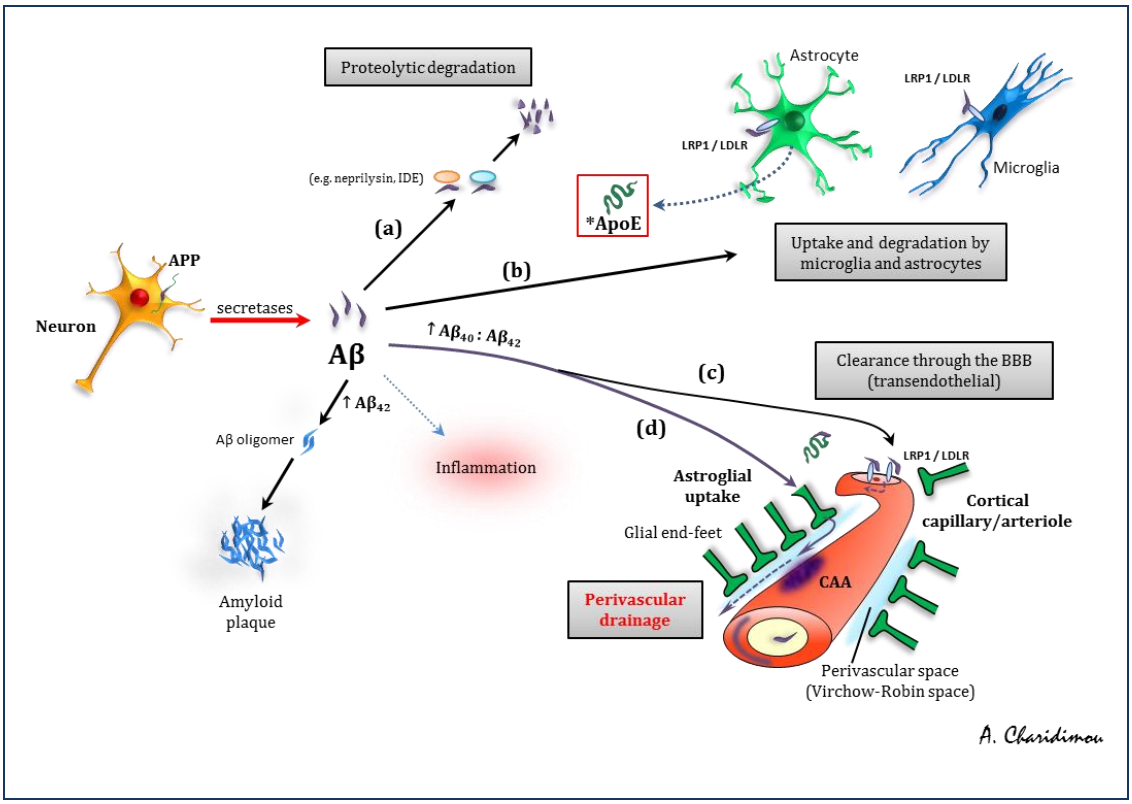

Figure 1.5 (A) Amyloid-β production, elimination and deposition in cerebral amyloid angiopathy (CAA). Converging evidence indicates that the major source of Aβ is neuronal. It is generated by sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretases, in proportion to neuronal activity. Aβ is eliminated from the brain by four major pathways: (a) proteolytic degradation by endopeptidases [such as neprilysin and insulin-degrading enzyme (IDE)]; (b) receptor-mediated clearance by cells in the brain parenchyma (microglia, astrocytes and to a lesser extent neurones; (c) active transport into the blood through the blood-brain barrier (BBB); (d) elimination along the perivascular pathways by which interstitial fluid drains from the brain.[86, 87] Specialized carriers (e.g. ApoE) and/or receptor transport mechanisms (e.g. the low-density lipoprotein receptor [LDLR] and LDLR-related protein [LRP1]) are involved in all major cellular clearance pathways. Vascular deposition is facilitated by factors that increase the Aβ40:Aβ42 (while increased Aβ42 leads to oligomerisation and amyloid plaques) and impede perivascular passage. As the clearance mechanisms fail with age, Aβ is increasingly entrapped from the perivascular drainage pathways into the basement membranes of capillaries and arterioles of the brain leading to CAA.

Evidence is emerging that cerebrovascular disease may impede the drainage along the perivascular pathways, contributing to CAA pathogenesis.[127, 131] It has been suggested that amyloid-β deposition could further impair/block the perivascular drainage, leading to dilation of perivascular spaces (also known as Virchow-Robin spaces) not only within lobar regions, but also in the underlying white matter, that itself shows is unaffected by CAA.[132, 133] These enlarged perivascular spaces can reach several millimetres in diameter and may be visible on appropriate brain imaging; this requires further investigation as a potential useful neuroimaging marker of CAA.[30, 133]

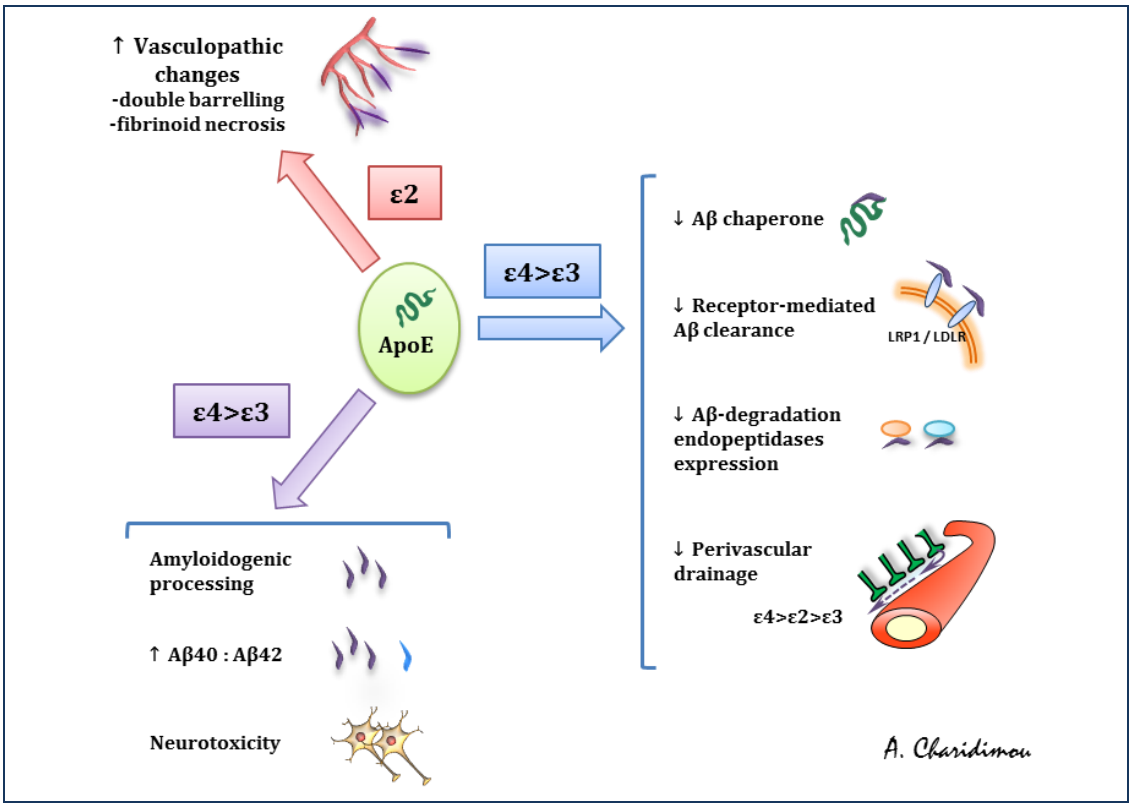

Figure 1.6 APOE alleles have a differential effect on different molecular and cellular processes of Aβ production, elimination and deposition in a way that they either increase or decrease the risk of developing CAA (B). The roles of different ApoE alleles in various pathways in the brain which might contribute in the pathogenesis and pathogenicity of CAA are summarised in (B).

As we have seen, APOE is a strong genetic risk factor for CAA, an effect mediated by its important role in amyloid-β metabolism, aggregation and clearance (Figure 1.6).[77, 125, 126]. APOE ε4 increases the amyloid-β_{40}:amyloid-β_{42} ratio shifting amyloid deposition to the vessels instead of brain parenchyma,[124] and may reduce the efficiency of efflux of amyloid-β along perivascular channels,[38, 134] influencing CAA risk and age of onset.[77, 135] APOE genotype may also interact with other small vessel disease changes: hypertensive arteriopathy, which leads to stiffening of the vessel wall, may reduce the pulsatile driving movements required for efficient perivascular drainage, and thus contribute to the risk of CAA.[38]

1.5.2 From amyloid-β deposition to CAA pathogenesis

Amyloid-β deposition has complex effects on vascular structure and function, which can result in brain injury.[2, 136] Important morphological changes include: loss of smooth muscle cells;[137] vessel wall thickening and lumen restriction;[101] endothelial dysfunction; and a loss of compliance leading to brittle, fragile vessels prone to microaneurysm formation and

leakage.[38] Acute trigger factors, for example sudden increases in blood pressure, or minor trauma (regularly encountered in clinical practice, but not to our knowledge formally studied), may cause the rupture of these abnormally weak, amyloid-laden vessels. Amyloid-β deposition may also impair local regulation of cerebral blood flow,[136] neurovascular unit function[138] and general homeostatic mechanisms in the ageing brain.[73] Other effects of vascular amyloid-β, including BBB disruption and active inflammation, could also contribute.[38, 136] Moreover, even without vascular deposition, soluble amyloid-β can cause abnormal vascular reactivity,[136] and induce the activation of inflammatory mediators including matrix metalloproteinase-9 and -2.[38, 139, 140]

1.6 The expanding clinical spectrum of sporadic CAA

There are at least four important clinical presentations associated with CAA:

- Symptomatic intracerebral haemorrhage
- Cognitive impairment and dementia
- Rapidly progressive cognitive and neurological decline (inflammatory CAA)
- Transient neurological symptoms

1.6.1 Intracerebral haemorrhage

1.6.1.1 The association between CAA and ICH

CAA is most often recognized in life by symptomatic, spontaneous, lobar ICH in elderly patients. The majority of ICHs (>75%) in the elderly are classified as spontaneous (sometimes also termed primary or non-traumatic), resulting from rupture of small arteries affected by two main processes: hypertensive arteriopathy or CAA. Hypertensive arteriopathy - characterized by lipohyalinosis and fibrinoid necrosis of small lenticulostriate arterial perforators - is considered an important cause of spontaneous ICH in deep or infratentorial locations (basal ganglia, thalamus, and pons). By contrast, CAA-related ICHs preferentially affect cortical-subcortical (lobar) regions (especially the occipital and temporal lobes[141]), less commonly the cerebellum, and rarely deep or brainstem structures, reflecting the distribution of the underlying microangiopathy.[37, 69, 109] The predilection for the occipital lobes is not well understood, but one hypothesis is that greater tortuosity of occipital small arteries impairs perivascular drainage.[38]

Clinicopathological studies suggest that CAA-related ICH accounts for at least 5-20% of all spontaneous ICH,[37, 56, 65, 72, 142, 143] and that the link is strongest for lobar ICH.

However, there are methodological challenges in attributing ICH to CAA: most pathological case-control studies did not systematically control for potential confounding risk factors for CAA, including cognitive impairment, ethnicity, or age. Furthermore, pathological studies showed differences in the prevalence of ICH only when comparing the presence of low grade CAA versus moderate-to-high grade CAA,[62, 65, 72, 101, 144-147] suggesting that mild CAA may not confer such a high risk of ICH. Since many elderly individuals in population-based studies have subclinical CAA without haemorrhage, CAA (especially if mild) may not be a sufficient cause of lobar ICH alone, but may interact with other factors, for example hypertension, neurodegenerative pathology or the use of anticoagulant drugs.[62, 65, 72, 101, 144-147]

1.6.1.2 Clinical features of CAA-related ICH

CAA-related ICHs have some distinct neuroimaging features, which are shown in Figure 1.7.[109, 148] However, the clinical presentation of CAA-related ICH is similar to other forms of lobar ICH (e.g. due to tumours or arterio-venous malformations) and varies according to ICH size and location. Patients usually present with an acute stroke syndrome with focal neurological deficits that may be associated with headache, nausea, vomiting, seizures and/or altered level of consciousness (especially large lobar bleeds).[45] There may also be a history of apparently minor head trauma, which might predispose to ICH in individuals with CAA. The typical lobar location of haemorrhage more often leads to acute seizures than in deep ICH. A first-ever ICH due to CAA may be relatively mild clinically, but this is counterbalanced by the high risk of recurrent haemorrhages; indeed, subsequent ICH (which characteristically may cluster over a short period of time [days to weeks]) is often much more severe.[149] In the longer term, survivors of lobar ICH are at higher risk of recurrence compared to deep ICH, with a rate of about 10% per year in elderly cohorts.[37, 149] Recurrent haemorrhages are typically lobar, often in the same lobe as the initial CAA-related bleed.[141] Multiple simultaneous lobar haemorrhages are characteristic of CAA-related ICH. The recovery from lobar ICH is often poor: negative prognostic factors include older age[84] and larger haematoma size;[150] conversely, a small superficial ICH without intraventricular extension is associated with better outcome.

1.6.1.3 Anticoagulant-related haemorrhage

CAA may be an important risk factor or cause for ICH related to oral anticoagulation use. Over the last decade there has been a five-fold increase in the incidence of anticoagulant-related ICH, which now accounts for about 15% of all ICH.[151] This trend is probably due to increasing use of warfarin to prevent cardioembolic stroke in elderly patients with atrial fibrillation. Anticoagulant use per se should not cause ICH if cerebral vessels are intact, but

the presence of CAA, rendering vessels brittle and fragile, is a plausible aggravating factor for such haemorrhage; an otherwise innocuous minor and self-limiting vessel leak (e.g a cerebral microbleed [CMB], see below) could form a life-threatening haematoma if the leaking vessel is damaged by advanced CAA. Evidence supporting a link between CAA and anticoagulation-related ICH includes the following observations: first, most such ICH occur with international normalised ratios within the therapeutic range[152] suggesting that an intrinsic disorder of cerebral small vessels could be important; and second, the APOE ε2 allele is more common in warfarin-related ICH than in patients on warfarin without ICH, supporting a role for CAA.[152] Although CAA may underlie a substantial proportion of anticoagulation-related haemorrhages, prospective studies with reliable diagnosis of CAA in life (e.g. by MRI evidence of lobar cerebral microbleeds or molecular imaging) in cohorts of patients treated with anticoagulants are urgently needed to answer this question (one large prospective MRI study is currently underway in the UK: www.ucl.ac.uk/cromis-2).

CAA may also be a risk factor for ICH after thrombolysis: spontaneous CAA-related and thrombolysis-related haemorrhages share some features including a predilection of lobar brain regions, multiplicity of haemorrhages, age-dependency and an association with dementia and leukoaraiosis.[153] In one small study, 2 of 5 cases of ICH after thrombolysis for acute myocardial infarction had severe CAA identified.[154]

1.6.2 Cognitive impairment and dementia

There is now increasing evidence that CAA is an important contributor to cognitive impairment,[10, 106] although dissecting its independent cognitive impact is confounded by the presence of co-existing Alzheimer's disease and other age-related pathologies (e.g. hypertensive arteriopathy). Nevertheless, in population-based clinical-pathological studies, the prevalence of CAA is consistently higher in demented compared to non-demented patients.[58] In the population-based Medical Research Council Cognitive Function and Aging Study (MRC CFAS) CAA was significantly associated with dementia (OR 9.3, 95% CI 2.7–41.0) even after controlling for age and dementia-related neuropathologies (e.g. neuritic and diffuse plaques).[61] Similarly, the HAAS autopsy study revealed a significantly higher prevalence of severe CAA in demented versus non-demented patients (43% vs. 24%).[62] CAA may worsen the severity of cognitive dysfunction in Alzheimer's disease: CAA together with Alzheimer's disease pathology has been associated with significantly worse cognitive performance during life, compared to Alzheimer's disease alone, even after controlling for age, neurofibrillary tangles and amyloid plaques number, infarctions and APOE genotype.[62] There are few studies of the specific pattern of cognitive impairment

associated with CAA; a recent autopsy series found that moderate-to-severe CAA (present in 19% of the study population) was associated with lower performance in specific cognitive domains, notably perceptual speed and episodic memory, after accounting for Alzheimer's disease pathology and other potential covariates.[155] The pathophysiological mechanisms by which CAA could cause cognitive impairment have not been well established,[10] but relevant lesions on brain imaging could include cerebral microbleeds,[156] microinfarcts,[73, 157] and white matter changes.[158]

CAA is thus emerging as a potentially important link between neurodegenerative and cerebrovascular pathology.[159] Vascular cognitive impairment and Alzheimer's disease are now conceptualised as a continuum[10, 160, 161] with complex interactions and shared risk factors.[136, 159] CAA seems likely to exacerbate the deleterious effect of neurodegenerative pathology on the brain, lowering the threshold for overt dementia.[10, 136] Unravelling the independent contribution of CAA to cognitive function is particularly important as it could lead to new therapeutic strategies.

1.6.3 Rapidly progressive cognitive and neurological decline: CAA-related inflammation

CAA is clearly a direct cause of cognitive impairment in the uncommon but clinically striking presentation of CAA-related inflammation (also termed cerebral amyloid angiitis, amyloid beta related angiitis and cerebral amyloid inflammatory vasculopathy).[162] CAA-related inflammation typically affects older adults, who present with acute to subacute cognitive decline, headache, behavioural change, seizures and focal neurological deficits.[162] Typical MRI findings include patchy or confluent, asymmetric T2-weighted or FLAIR white matter hyperintensities (with or without mass effect and leptomeningeal or parenchymal enhancement).[162] T2*-weighted gradient recalled echo (T2*-GRE) or susceptibility weighted imaging (SWI) may reveal previous lobar haemorrhage and/or multiple cortical and subcortical microbleeds.[162] The major differential diagnoses include infections (in particular progressive multifocal leucoencephalopathy), neuro-sarcoidosis, immune-related conditions (e.g. acute disseminated encephalomyelitis)[163] and malignancies.[162] Definite diagnosis requires brain and leptomeningeal biopsy showing perivascular inflammation with mononuclear or multinucleated giant cells associated with amyloid-β-laden vessels and/or frank vasculitis.[162] Although the clinical course of CAA-related inflammation is varied, it is important to recognize because it may respond well to immunosuppressive treatment (e.g. high dose corticosteroids or cyclophosphamide).[162, 164] This distinct syndrome has parallels with that observed in patients with Alzheimer's disease who developed meningo-

encephalitis after immunization against human Amyloid-β, where post mortem examination revealed inflammation and/or vasculitis associated with CAA.[165, 166]

1.6.4 Transient focal neurological episodes

After ICH, the next most commonly described clinical presentation of sporadic CAA is with transient neurologic episodes,[167-169] sometimes termed "amyloid spells". The most common type of attack involves recurrent, stereotyped episodes of "positive" spreading sensory symptoms (paraesthesias).[167, 168] Although there are a number of small case reports and series,[167, 168, 170, 171] no large systematic studies have investigated the prevalence or semiology of these phenomena. At least two other types of transient events have been described: partial motor seizure-like episodes (e.g. limb shaking); and visual disturbances (usually positive visual symptoms similar to migrainous auras). Spells are typically brief, almost always less than about 30 minutes, and usually less than a few minutes. The attacks seem likely to be related to haemorrhagic components of CAA: associated neuroimaging findings reported include cerebral microbleeds and convexity subarachnoid haemorrhage (cSAH) in the cortical region corresponding to the spell.[167, 171] The diagnosis of these CAA-related attacks is of clinical relevance, since they seem to precede serious symptomatic ICH in some patients; antiplatelet or anticoagulant use following such an attack misdiagnosed as a transient ischaemic attack (TIA) could therefore cause potentially avoidable intracranial bleeding. The underlying mechanisms of CAA transient spells remain unclear, but could include seizure-like activity (perhaps related to small areas of bleeding, e.g. microbleeding, cSAH or superficial siderosis); a direct effect of amyloid or bleeding on local cortical function; or spreading cortical depression.[167] The responsiveness of these attacks to anti-epileptic drugs as well as their spreading nature in many of the reported cases are in favor of a seizure-like mechanism for their pathophysiology. In a case series by Roth and co-workers,[168] four out of six patients with these transient attacks responded to anticonvulsants, while the other two patients showed improvement after the cessation of antiplatelet therapy. Typical TIA-like episodes have also been reported in CAA,[169] but whether these are genuinely due to ischaemia and should be treated with antithrombotic agents requires further study.

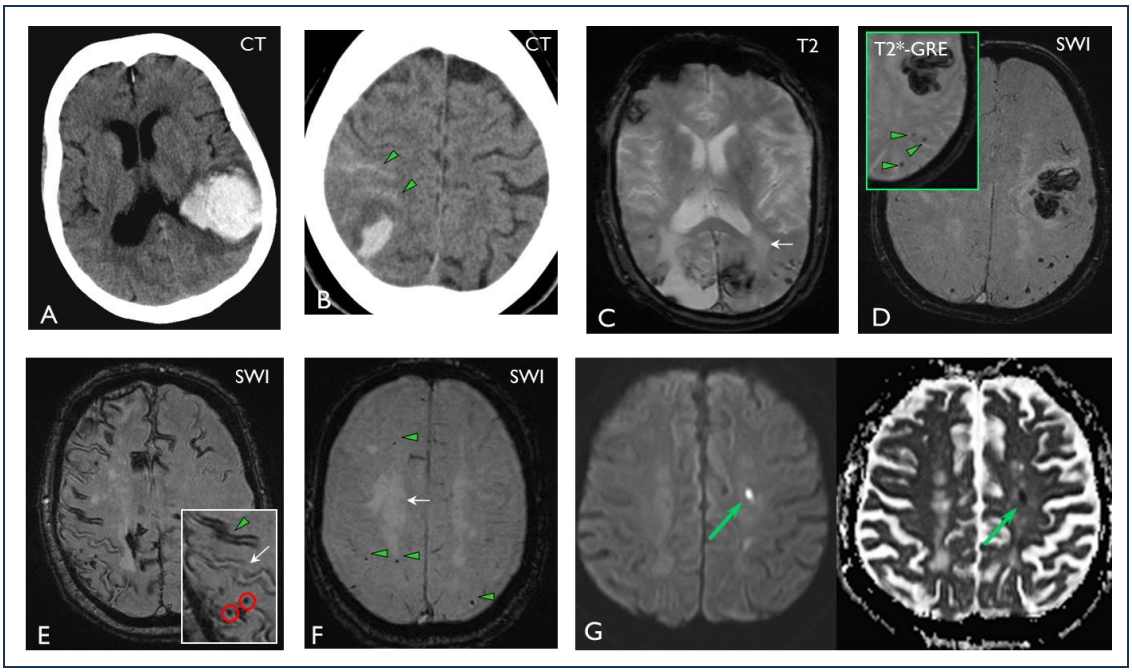

Figure 1.7 The spectrum of imaging manifestations of sporadic cerebral amyloid angiopathy (CAA) . (A) An acute lobar haematoma on computer tomography (CT) scan. Some extension of the bleeding in the posterior horn of the left ventricle can be seen. (B) CT scan of a patient with a small posterior cortical haematoma. Acute cortical subarachnoid haemorrhage (cSAH) is evident in two adjacent sulci (arrowheads). (C) A T2-weighted MRI of a patient with probable CAA showing two lobar foci of recent/subacute intracerebral haemorrhage (ICH): in the medial aspect of the left occipital lobe, and in the right inferior frontal gyrus. There is also a large old lobar haemorrhage involving the right occipital lobe, some scattered cortical-subcortical cerebral microbleeds (CMBs) in posterior brain regions, as well as confluent white matter hyperintensities (leukoaraiosis: arrow). (D) SWI and T2*-GRE (inset) done on the same day in a patient with a large lobar bleed. The detection of strictly lobar CMBs (better demonstrated on SWI) allowed the diagnosis of probable CAA. (E) cSAH (linear hypointensities in the subarachnoid space on T2*-GRE/SWI) and focal cortical siderosis (hyperintense on T2*-GRE/SWI). The inset in (E) demonstrates the co-existence of cSAH (arrowhead), focal cortical siderosis in an adjacent sulcus (arrow) and some CMBs (circles). Focal cortical siderosis represents the chronic lesion following an acute cSAH. (F) The use of SWI, in a patient presenting with progressive cognitive impairment let to the detection of multiple strictly lobar microbleeds, characteristic of CAA. Confluent white matter changes (arrow) are also visible. (G) Restricted diffusion (diffusion-weighted image on left, absolute diffusion-weighted imaging (DWI) on the right) consistent with a small acute ischaemic lesion in a patient with probable CAA.

1.7 Neuroimaging (MRI) correlates of CAA

The important MRI correlates of CAA (Figure 1.7) include:

- Cerebral microbleeds
- White matter hyperintensities (leukoaraiosis)
- Convexity subarachnoid bleeding
- Cortical superficial siderosis
- Acute ischaemic lesions (cerebral microinfarcts)

1.7.1 Cerebral microbleeds

The widespread use of T2*-weighted MRI sequences in the last decade or so has led to the increasing detection of cerebral microbleeds: small, well-demarcated, hypointense, rounded lesions, not detected on conventional MRI (Figure 1.7 D-f).[35]. Histopathological studies show that cerebral microbleeds correspond to focal accumulations of haemosiderin-laded macrophages (a blood-breakdown product) adjacent to abnormal small vessels affected by hypertensive angiopathy or CAA.[172, 173] There is increasing evidence that hypertensive vasculopathy is associated with cerebral microbleeds in deep brain regions (basal ganglia, thalamus and brainstem), whereas CAA is characterised by cerebral microbleeds in a lobar distribution[35, 173] with a predilection for the parietal lobes.[141] The Rotterdam scan study[174, 175] showed a strong association between strictly lobar (but not deep) cerebral microbleeds and APOE ε4, consistent with the well-known relation of this allele with CAA.[87] A recent imaging study in clinically probable CAA, using non-invasive amyloid imaging with Pittsburgh Compound B (PiB), found that cerebral microbleeds correspond to areas with a high concentration of amyloid.[176] Further evidence derives from neuroimaging studies of cerebral microbleeds in patients presenting with symptomatic ICH: patients with deep ICH, predominantly caused by hypertensive arteriopathy, are more likely to have deep cerebral microbleeds; whereas those patients with multiple strictly lobar ICH, frequently caused by CAA (although the exact proportion of lobar ICH which are actually due to CAA in not known), are more likely to have multiple strictly lobar cerebral microbleeds.[177-179] Moreover, cerebral microbleeds correlate with the risk of lobar ICH recurrence[180] suggesting an important role in prognosis (as well as diagnosis) in CAA.[27]

Recent population-based studies have revealed a high percentage of community-dwelling elderly people with strictly lobar microbleeds, (particularly in the posterior brain regions) suggesting sub-clinical CAA.[174, 175, 181, 182] This may have important implications: if strictly lobar cerebral microbleeds are validated as a diagnostic marker of CAA, such asymptomatic individuals could benefit from new therapeutic agents to reduce the progression of the disease.

Neuroimaging studies have revealed lobar cerebral microbleeds in more than 20% of patients with Alzheimer's disease,[183] probably reflecting advanced CAA (in keeping with neuropathological findings). Patients with autosomal dominant forms of familial Alzheimer's disease (who have a younger age at symptom onset), also seem to have a prevalence of lobar cerebral microbleeds similar to sporadic Alzheimer's disease[184] – a striking recent observation since these patients are much more likely to have 'pure' neurodegenerative Alzheimer's disease without co-existing sporadic small vessel disease. It has been suggested

that the presence of multiple lobar cerebral microbleeds in patients with Alzheimer's disease, may identify a specific subgroup of patients with a different clinical phenotype with therapeutic implications which need to be explored in future studies.[183]

1.7.2 White matter hyperintensities

White matter hyperintensities (also known as leukoaraiosis) is a radiological term which describes imaging changes (often confluent) in deep cerebral white matter. Leukoaraiosis appears as low attenuation on computer tomography (CT) scans or hyperintensity on T2-weighted or FLAIR MRI, typically sparing subcortical U-fibres (Figure 1.7 C).[5] Pathological substrates include demyelination, axon loss and mild gliosis. The pathogenesis of leukoaraiosis in CAA probably involves chronic hypoperfusion of the vulnerable peri-ventricular white matter and disruption of the BBB due to amyloid in the overlying cortical small vessels.[2, 24, 133] Another possible mechanism of leukoaraiosis in CAA is as a result of the accumulation of silent ischaemic lesions (microinfarcts).[46, 73, 185] Indeed, leukoaraiosis is very common in CAA, preferentially involving posterior regions,[186] although some studies suggest no major difference in the topography of leukoaraiosis in CAA compared to hypertensive arteriopathy.[158, 186] A recent investigation suggested that subjects with CAA-related lobar ICH have a higher prevalence of occipital dominant leukoaraiosis compared to normal elderly controls;[187] this interesting finding requires further clinical attention and investigation. Leukoaraiosis may be an important contributor to overall disease burden, especially progressive cognitive impairment,[188] given its tendency to accumulate over time.[189] A recent study found that leukoaraiosis volume was greater in patients with CAA and hypertension than those without, suggesting that strict control of hypertension might reduce leukoaraiosis-related disability in CAA.[188]

1.7.3 Acute convexity subarachnoid haemorrhage (cSAH) and cortical superficial siderosis

Atraumatic convexity subarachnoid haemorrhage (cSAH) and cortical superficial siderosis are recently recognised imaging correlates of sporadic CAA[190] which seem to be quite characteristic of the disorder (Figure 1.7 E). cSAH is localized bleeding, usually in up to several adjacent sulci, without other subarachnoid bleeding at the base of the brain in the pattern typically associated with saccular aneurysm rupture.[191] Although rare in isolation, in CAA, cSAH often results from lobar ICH extending to the cortical surface.[170, 190, 192] The largest cohort of patients with isolated cSAH published (n=29), found that CAA was a

frequent apparent cause in patients over 60 years old.[191, 193] A recent retrospective analysis of consecutive patients admitted to a tertiary stroke unit with cSAH suggested that CAA could be a common cause in the elderly, with a characteristic clinical presentation of single or recurrent transient focal neurological attacks.[171] Another recent study of a cohort of patients presenting with cSAH, reported similar findings.[194]

Superficial siderosis describes haemosiderin deposition in the superficial layers of the cerebral cortex, and may follow repeated episodes of bleeding in the subarachnoid space.[190] On T2*-GRE MRI sequences superficial siderosis shows a characteristic "gyriform" pattern of hypointense signal (Figure 1.7 E).[190] Linn et al. have recently detected superficial siderosis in 47.4% (n=38) of patients with a clinical diagnosis of CAA, compared to no controls (mean age, 54 years), suggesting that it might be helpful for the clinical diagnosis of CAA (see below).[195] Compared to the well-known syndrome of central nervous system superficial siderosis, which typically affects the brainstem and posterior fossa (associated with cerebellar and brainstem signs), CAA-related siderosis has a predilection for the cerebral convexity[196] and may be associated with transient neurological manifestations.[168]

1.7.4 Small acute ischaemic lesions on diffusion-weighted imaging (DWI)

Neuropathological evidence of asymptomatic ischaemic infarction (cerebral microinfarcts[197]) is an established finding in the brains of patients with advanced CAA.[43, 101-103] Recent studies using magnetic resonance DWI, -which is extremely sensitive to even small areas of acute ischaemia - have shed light on the dynamics of this phenomenon *in vivo* (Figure 1.7 G). A case report[47] and a recent case-control study[46] found a high prevalence of DWI-positive lesions, in patients with advanced CAA. These lesions were associated with CMB burden, suggesting shared pathophysiological pathways.[198] Gregoire et al recently established that acute, subclinical ischaemic brain lesions are frequent after recent acute ICH, and are three times more common in CAA-related ICH than other spontaneous bleeds;[73] the lesions were associated with the severity of leukoaraiosis and lobar cerebral microbleeds, suggesting that that they were due to a CAA-related occlusive arteriopathy.[73] However, a more recent larger study did not appear to show this higher prevalence of DWI lesions in CAA-related intracerebral haemorrhage, suggesting that both CAA and hypertensive arteriopathy trigger high rates of infarction.[199] These data suggest a dynamic interplay between the haemorrhagic ("microbleeding") and ischaemic ("microinfarction") components in CAA and small vessel disease in general,[198] though the therapeutic implications and prognostic significance of these findings require further study. The topic of

microinfarcts and DWI lesions in CAA as well as current uncertainties in the terminology and pathophysiology of these lesions, are further covered in Discussion (Chapter 11).

1.8 Molecular imaging of vascular amyloid in vivo

MRI indirectly detects the consequences of CAA (e.g. cerebral microbleeds, cSAH, and siderosis) rather than vascular amyloid itself. Consequently, a large proportion of "silent" CAA may be as yet undetectable. Positron emission tomography (PET) methods allow the *in vivo* imaging of amyloid in the brain, using several radioligands, of which the most widely studied is ^{11}C Pittsburgh Compound B (PiB).[200] Ly and colleagues demonstrated that CAA subjects had increased global PiB uptake relative to a healthy elderly control group, and found an occipital predominance of PiB retention in CAA compared with Alzheimer's disease.[50] PiB PET might therefore ultimately detect CAA *in vivo*, even before it causes symptomatic ICH or the known radiological sequelae including cerebral microbleeds.[48, 49, 176, 201]

1.9 Diagnostic approach to CAA: the critical role of neuroimaging

A common clinical scenario where sporadic CAA should be suspected is in elderly patients presenting with lobar ICH. The most commonly used criteria for CAA diagnosis are the Boston criteria.[202] In the absence of direct neuropathological examination, CAA is diagnosed based on characteristic neuroimaging findings.[202] The diagnosis of probable CAA requires (Table 1.2):

- Age >55 years
- the detection of multiple haemorrhagic cerebral lesions
- haemorrhages confined to cortical or cortical-subcortical (lobar) brain regions
- exclusion of secondary causes of ICH, such as arteriovenous malformations, head trauma, brain tumour, vasculitis and excessive anticoagulation

The specificity of the Boston criteria has been validated against the established gold standard of neuropathological diagnosis from autopsy, haematoma evacuation, or cortical biopsy.[202] In this study, the criteria showed excellent specificity: all cases identified as "probable CAA" (n=13) had pathological evidence of severe CAA. However, the sensitivity of the probable category was 44%, so that it failed to identify over 50% of those with

severe CAA pathology.[202] However, this study does not reflect current radiological practice since only 15 patients had T2*-GRE imaging. Recently, the application of the Boston criteria with a greater use of T2*-GRE MRI in Dutch-type hereditary CAA found a much improved sensitivity (especially when lobar cerebral microbleeds were included in the criteria).[203] The rationale for the inclusion of lobar cerebral microbleeds in the criteria is that both lobar cerebral microbleeds and lobar ICH represent independent vascular rupture events which are considered to offer equal evidence for the presence of CAA.[204] The recently introduced SWI, a three-dimensional T2*-GRE technique, enables visualization of cerebral microbleeds with much increased sensitivity, resulting in higher lesion counts (at least 67% more compared to conventional T2*-GRE) (Figure 1.7 D),[205-207] but its effect on diagnostic accuracy for CAA requires further study. Superficial siderosis and cSAH, which have a high prevalence in CAA-related ICH but are rare in other forms of ICH, have been shown to enhance the sensitivity of the Boston Criteria without loss of specificity.[195]

Table 1.2 Classic and modified Boston criteria for diagnosis of cerebral amyloid angiopathy (CAA). (*Modifications compared to the classic Boston criteria based on Linn et al.)

1. Definite CAA
Full post-mortem examination demonstrating:
- Lobar, cortical, or cortical-subcortical haemorrhage
- Severe CAA with vasculopathy
- Absence of other diagnostic lesion

2. Probable CAA with supporting pathology
Clinical data and pathologic tissue (evacuated haematoma or cortical biopsy) demonstrating:
- Lobar, cortical, or cortical-subcortical haemorrhage
- Some degree of CAA in specimen
- Absence of other diagnostic lesion

3. Probable CAA
Clinical data and MRI or CT demonstrating:
- Multiple haemorrhages restricted to lobar, cortical, or cortical-subcortical regions (cerebellar haemorrhage allowed)
  **[OR single lobar, cortical, or cortical-subcortical haemorrhage and focal[b] or disseminated[c] superficial siderosis]*
- Age≥55 years
- Absence of other cause of haemorrhage[a]

4. Possible CAA
Clinical data and MRI or CT demonstrating:
- Single lobar, cortical, or cortical-subcortical haemorrhage
  **[OR focal[b] or disseminated[c] superficial siderosis]*
- Age ≥55 years
- Absence of other cause of haemorrhage[1]

Other causes of haemorrhage (differential diagnosis of lobar haemorrhages):
Antecedent head trauma, haemorrhagic transformation of an ischaemic stroke, arteriovenous malformation, haemorrhagic tumour, warfarin therapy with international normalisation ratio > 3, vasculitis

[b] **Focal siderosis: siderosis restricted to 3 or fewer sulci**
[c] **Disseminated siderosis: siderosis affecting at least 4 sulci**

Although the value of T2*-weighted MRI and SWI to detect cerebral microbleeds, cSAH and siderosis has mainly been validated in cohorts of patients who presented with symptomatic ICH, such imaging may also have a role in the diagnosis of patients presenting without major haemorrhage, but with other syndromes raising suspicion of CAA; for example, elderly patients with progressive cognitive impairment.[10, 171, 206, 208, 209] In addition, although at present T2* MRI or SWI sequences are not part of the routine investigation of TIA-like attacks, there might be useful in patients with CAA-related transient focal neurological episodes ("amyloid spells") – rather atypical of TIAs.[168, 171] However, current data are insufficient to make evidence-based recommendations.

Other biomarkers might also prove useful in the non-invasive diagnosis of CAA, in particular the assessment of amyloid-β concentrations in the cerebrospinal fluid (CSF). Decreased levels of CSF amyloid-β_{42} but not amyloid-β_{40} are found in Alzheimer's disease,[210] while it has been reported that both amyloid-β_{42} and amyloid-β_{40} concentrations are decreased in CAA, relative to control and Alzheimer's disease patients.[211] It has also been suggested that the combination of low amyloid-β_{42} with increased total tau levels in the CSF, can discriminate CAA patients from normal controls with high accuracy.[211] Another potentially promising marker of CAA might include retinal changes (microaneurysms and dot and blot haemorrhages[212]). A critical goal of all of these potential approaches is to reliably identify CAA at the early (asymptomatic) stages of the disease, to allow the best chance for disease modifying or preventive treatments to be effective.

1.10 Management of CAA and its consequences

1.10.1 Acute treatment

No treatment is specific for symptomatic management of CAA or CAA-related ICH. As in all forms of spontaneous ICH, CAA-related haematomas enlarge in the first few hours after onset, providing a potential target for treatment. One of the most promising available treatments in acute ICH is intensive lowering blood pressure, which has been shown to reduce haematoma expansion in a randomized trial,[213] presumably by reducing pressure into the ICH in the critical hyperacute phase. A further large study (INTERACT-2) showed that intensive blood pressure lowering in acute ICH patients did not result in a reduction in the rate of death or severe disability (a score of 3 to 6 on the modified Rankin scale). However, an ordinal analysis of modified Rankin scores indicated improved functional outcomes in the patient group with intensive lowering of blood pressure.[214] The role of

neurosurgery in ICH is a topic of ongoing investigation. Although there have previously been concerns regarding surgery in CAA due the risk of bleeding from fragile amyloid-laden vessels, the available evidence does not suggest a particularly high operative risk.[215-217] Neurosurgery for haematoma evacuation appears relatively safe in at least some patients with CAA-related ICH, particularly in patients under 75-year old without intraventricular extension.[217] This topic was recently explored in the STICH-2 trial (early surgery versus initial conservative treatment in patients with spontaneous supratentorial lobar intracerebral haematomas). STICH-2 confirmed that early surgery does not seem to increase the rate of death or disability at 6 months and might actually have a small but clinically relevant role in patients with spontaneous superficial ICH without intraventricular haemorrhage.[218] Minimally invasive surgery plus recombinant tissue-type plasminogen activator (MISTIE trial) is also an area under investigation.[219] Until further evidence for specific acute treatments is available, it is reasonable to follow the American Heart Association Stroke Council guidelines for acute management of ICH, without modification for individuals with suspected CAA.[220] Active research into new approaches for acute ICH treatment is expected to benefit patients with CAA-related bleeds. Novel approaches including neuroprotective drugs[221, 222] which target the multitude of processes that occur after ICH (e.g. cerebral edema, thrombin release, red blood cell lysis and haemoglobin-induced neurotoxicity),[223, 224] and iron-chelating agents (such as deferoxamine)[225] are all being studied in early-phase trials.[44, 220, 226]

1.10.2 Prevention of recurrent ICH

1.10.2.1 Withholding anticoagulants and antiplatelets

It is a paradox that many elderly patients at highest risk of occlusive vascular events are also at the highest risk of haemorrhage complications including ICH. Judging the balance of risk and benefit of antithrombotic treatment after ICH in those patients with an indication for vascular secondary prevention is thus a major clinical challenge. The available evidence on this topic is limited, consisting of generally small case-control and prospective observational studies. In a recent prospective cohort of patients with spontaneous lobar ICH, an association was found between aspirin use and ICH recurrence after adjusting for other potential ICH risk factors (HR 3.95, 95% CI 1.6–8.3, p <0.021).[227] Re-bleeding risk was associated with the number of lobar cerebral microbleeds, and the presence of leukoaraiosis in posterior brain regions-possible markers of underlying CAA and its severity.[227] Gregoire et al. in a small case-control study found that lobar cerebral microbleeds were associated with antiplatelet-related ICH, also supporting a link between

CAA and antiplatelet-related ICH.[228] Another small case-control study of warfarin-related ICH and matched ICH-free warfarin users showed an association of warfarin with cerebral microbleeds, but with large confidence intervals around the odds ratios for the association.[212] There are no randomized trial data, but a decision analysis suggested that in patients with CAA-related ICH, the use of anticoagulants to prevent future cardioembolic (atrial fibrillation-related) stroke would lead to an ICH rate that outweighs any benefit from the treatment.[229]

For the moment, anticoagulation should usually be avoided in patients with a diagnosis of CAA and symptomatic lobar ICH, unless there is a very compelling need to treat that could outweigh the very high risk of recurrent ICH (e.g. life threatening pulmonary embolism or a mechanical heart valve). Although antiplatelet drugs probably also increase future ICH risk in CAA, it may be reasonable to consider them in selected patients with CAA for secondary prevention in whom the risk of intracerebral bleeding is judged to be low and the risk of occlusive vascular events high, based in their clinical and imaging characteristics. In primary prevention the risk/benefit ratio may favour withholding treatment in patients with multiple lobar cerebral microbleeds. Further randomized clinical trials are urgently needed to help clarify the optimum antithrombotic treatment in these different CAA patient groups.

1.10.2.2 Blood pressure control

A recent subgroup analysis of the PROGRESS trial reported that lowering blood pressure with the antihypertensive drug perindopril (with or without indapamide) reduced the risk of probable CAA-related ICH by 77% (95% CI, 19%–93%) over a follow-up period of 3.9 years.[76] Despite a small total number of CAA-related ICH events, this is the first trial to show that blood pressure-lowering treatment protects against CAA-related ICH, regardless of the presence of hypertension.[76] Blood pressure lowering may also be associated with a more general benefit in cardiovascular risk and mortality in patients over the age of 80 years.[230] Thus, most patients with CAA and a history of symptomatic ICH should be offered antihypertensive treatment, but further trials are clearly needed.

1.10.2.3 Statins

Recently, concerns have been raised over statins as a risk factor for ICH, in light of the results of the SPARCL trial of atorvastatin in patients with stroke, which showed a small increase in the incidence of ICH among patients receiving high doses of the drug;[231] the hazard was higher for patients with baseline haemorrhagic compared to ischaemic stroke (HR 4.1 *versus* 1.6).[232] A decision analysis showed that the risk of statin therapy likely

outweighs any potential benefit in patients with recent lobar ICH.[233] Thus, although there are inadequate data and a hot debate for clear recommendations on statin use,[220, 234, 235] they should perhaps be avoided in the setting of a recent CAA-related ICH.[236] For individuals with suspected CAA based on the presence of multiple lobar cerebral microbleeds (without any associated macro-bleeding) the risks and benefit of statin therapy are uncertain[27] and randomised trials are needed.

1.11 Conclusions

During the last decade, there have been tremendous advancements in our understanding of CAA, relating to its pathophysiology, clinical spectrum, imaging manifestations and diagnosis.

- Sporadic CAA is a common disease of the elderly and will become an increasingly important healthcare challenge as populations age further.
- Sporadic CAA is an important contributor to cognitive decline and spontaneous or anticoagulant-related lobar ICH.[152]
- Transient neurological spells in CAA may be misdiagnosed as TIAs, but seem to have characteristic clinical features; they need to be recognized since treating them with antithrombotic drugs may increase the risk of future ICH.
- Recent advances in neuroimaging have provided a new imaging window into the dynamic haemorrhagic and ischaemic features of CAA.
- Lobar cerebral microbleeds, cSAH and cortical superficial siderosis show promise to reliably diagnose CAA in life, though validation of these findings against their histopathological correlates requires further study.
- Molecular imaging of amyloid-β may further improve our ability to detect this condition in vivo and define its true prevalence and burden.[48-50, 176]
- The rapidly developing field of transgenic mouse modelling has provided significant insights into the pathophysiology of human CAA, including the key pathogenetic role of the perivascular drainage pathway and the differential effects of different APOE genotype.[38]

Despite our improved understanding of CAA, there are still many questions to be answered in order to identify targets for therapeutic and preventive interventions. Exciting diagnostic and therapeutic developments are on the horizon for this fascinating small vessel disorder.

Aims and rationale of this PhD thesis

The detection of CAA during life is becoming an increasingly important challenge, since approaches of prevention or treatment (disease-modification) are now emerging as realistic possibilities, including reducing amyloid-β production, clearing amyloid-β using immune-based therapies or protecting small vessels from its toxic effects (covered in Chapter 11).[1] Determining the most promising treatments requires development of reliable biomarkers, the goal of my research. A biomarker is a measurable characteristic that reflects the presence or severity of a disease. Biomarkers may predict susceptibility to disease, improve understanding of mechanisms, improve the accuracy of diagnosis, or track progression, including treatment effects (when they are often termed "surrogate" markers); they may be based on clinical factors (e.g. clinical events), structural neuroimaging lesions, biological samples (blood, cerebrospinal fluid, genetic markers), or in the case of CAA, markers of vascular pathophysiology and direct amyloid-β radioligands.

The main objective of this PhD thesis is to provide new insights into potential clinical and applied clinical neuroimaging biomarkers in patients with CAA. This is accomplished by a portfolio of research studies.

Chapter 2 explores the clinical and radiological spectrum of transient focal neurological episodes as a potential clinical clue for CAA. This is a poorly characterised phenotype of CAA with important clinical relevance.

Chapters 3 and 4 focus on cortical superficial siderosis as both a diagnostic and a prognostic marker of CAA respectively. Implications for mechanims are also explored.

Chapters 5 to 7 are dedicated to MRI-visible perivascular spaces, a recently recognized (or rediscovered) potential neuroimaging marker of small vessel disease,[30, 133] which has often been ignored. **Chapter 5** focuses on the imaging characteristics of perivascular spaces in relation to spontaneous ICH with the aim to determine the prevalence, distribution and severity of perivascular spaces in ICH, and investigate associations with other imaging markers of small vessel disease, and with ICH aetiology. In **Chapter 6**, results from an MRI-neuropathological study exploring MRI-visible perivascular spaces in the centrum semiovale (i.e. cerebral white matter) as a diagnostic marker of pathology-confirmed CAA are presented. **Chapter 7** describes the relationship between centrum semiovale perivascular spaces and cerebrovascular amyloid-β burden in a PiB-PET-MRI study across a range of CAA severity.

Chapter 8 discusses a novel association between white matter perivascular spaces and cortical superficial siderosis in CAA patients, and its potential pathophysiological importance.

Chapter 9 explores potential pathological, neuroimaging and genetic differences in patients with pathology-proven CAA with and without intracerebral haemorrhage and presents evidence for different disease phenotypes.

Chapter 10 is a systematic review and meta-analysis which assess the evidence whether the presence and burden of cerebral microbleeds on MRI scans is associated with an increased risk of recurrent spontaneous ICH, and if this risk is different according to MRI-defined microangiopathy subtype.

This thesis concludes in **Chapter 11** with a very brief review of the main findings, a discussion in the context of current knowledge and the description of further research plans in the field, including prospects for disease modifications in CAA.

Although overlap has been minimized as far as possible, each chapter is designed to stand alone, so some repetition between chapters is inevitable and necessary.

Chapter 2 Clinical-radiological spectrum of transient focal neurological episodes in cerebral amyloid angiopathy: multicentre MRI cohort study and meta-analysis

ABSTRACT

Objective: Transient focal neurological episodes (TFNE) are recognized in cerebral amyloid angiopathy (CAA) and may herald a high risk of intracerebral haemorrhage (ICH). We aimed to determine their prevalence, clinical-neuroimaging spectrum and association with future ICH risk.

Methods: Multicentre retrospective cohort study of 172 CAA cases. Clinical, imaging and follow-up data were collected. We classified TFNE into: (1) predominantly positive symptoms ("aura-like" spreading paraesthesias/positive visual phenomena; or limb jerking); and (2) predominantly negative symptoms ("TIA-like" sudden-onset limb weakness, dysphasia, or visual loss). We pooled our results with all published cases identified in a systematic review.

Results: In our multicentre cohort, 25 patients (14.5%; 95% CI: 9.6%-20.7%) had TFNE. Positive and negative symptoms were equally common (52% vs. 48% respectively). The commonest neuroimaging features were: leukoaraiosis (84%); lobar ICH (76%); multiple lobar cerebral microbleeds (58%); and superficial cortical siderosis/convexity subarachnoid haemorrhage (54%). CAA patients with TFNE had a significantly higher prevalence of superficial cortical siderosis compared to those without TFNE (50% vs. 19%; p=0.001.). Over a median period of 14 months, 50% of TFNE patients had a symptomatic lobar ICH. The meta-analysis showed a risk of symptomatic ICH of 25.3% (95% CI: 16.3%-38.0%) at 8 weeks, related neither to clinical features, nor previous symptomatic ICH.

Conclusions: TFNE are common in CAA, and include both positive and negative neurological symptoms. TFNE are associated with superficial cortical siderosis and predict a high early risk of symptomatic ICH (which may be amenable to prevention). Blood-sensitive MRI sequences are important in the investigation of such episodes.

2.1 Introduction

Sporadic cerebral amyloid angiopathy (CAA) is a common age-related cerebral small vessel disease characterised by the progressive deposition of amyloid-β in the wall of cortical and leptomeningeal small arteries.[28] CAA is a common cause of spontaneous lobar intracerebral haemorrhage (ICH) and cognitive impairment in the elderly.[28]

Another characteristic clinical presentation associated with CAA is with transient focal neurologic episodes (TFNE), sometimes termed "amyloid spells".[167-169] Most published cases describe recurrent, stereotyped, spreading paraesthesias, usually lasting several minutes.[167, 168] The recognition of these episodes is of clinical importance because: (a) they may have diagnostic value as the most common clinical presentation of CAA other than ICH; and (b) they may precede symptomatic ICH,[237] a risk which could be reduced, for example by avoiding antithrombotic use following misdiagnosis of such an episode as a typical transient ischaemic attack (TIA). The available evidence on TFNE associated with CAA consists only of case reports and small case-series (<10 patients),[167, 168, 170, 171, 194] which may be subject to publication bias, limiting their generalizability.

In the present study our aims were to determine the prevalence, clinical features, neuroimaging correlates and future ICH risk associated with CAA-related TFNE, using data from a multicentre CAA cohort. We hypothesised that TFNE are common in CAA and that they signify a high risk of future symptomatic ICH. We pooled our results with all previously published studies identified in a systematic review.

2.2 Methods

2.2.1 Participants

We included patients diagnosed with CAA at four stroke centres in the United Kingdom and Belgium over defined time periods. The hospitals were: University College London Hospitals NHS Foundation Trust (London) (03/2003–09/2011), Addenbrooke's Hospital (Cambridge) (07/2002–03/2010), Cliniques Universitaires Saint Luc (Brussels) (12/2003–04/2010) and Université Catholique de Louvain (08/2005–03/2009). At all participating centres, MRI scanning is a routine investigation for all cases of suspected CAA, unless there are contra-indications. Our inclusion criteria were: (1) possible, probable or definite CAA, defined according to the Boston criteria;[202] and (2) a clearly documented history of transient (≤24 hours), fully resolving, focal neurological episodes with no known alternative

explanation other than CAA (e.g. structural brain lesion, atrial fibrillation, extracranial or intracranial stenosis). We classified TFNE into two categories: (a) predominantly positive focal symptoms ("aura-like" spreading paraesthesias, positive visual phenomena or limb jerking); and (b) predominantly negative focal symptoms ("TIA-like" sudden-onset limb weakness, dysphasia, or visual loss). We excluded patients without an adequate medical history or imaging, those not meeting the criteria for CAA, and those with non-focal transient symptoms (e.g. generalized seizures, confusion, and disorientation).

2.2.2 Data collection

Cases were ascertained using multiple overlapping methods from prospective clinical databases and radiological reports; 172 patients with possible (n=54), probable (n=115), probable with supportive pathology (n=2) or definite (n=1) CAA were included. Two patients were excluded because of an alternative explanation of TFNE (one with significant carotid stenosis, one with sepsis), two because an adequate history was not available, and 11 because episodes were not focal.

Demographic and clinical information were collected using standardised data collection forms. Follow-up information on recurrent cerebrovascular events (including ICH) was obtained from prospective databases and medical records.

2.2.3 Magnetic resonance imaging acquisition and analysis

The MRI protocol was standardized in each hospital. Imaging was at 1.5T field strength for all patients and included T1-weighted, T2-weighted, fluid attenuated inversion recovery (FLAIR), and T2*-weighted gradient-recalled echo (T2*-GRE) sequences. For some patients susceptibility-weighted imaging (SWI) and diffusion-weighted imaging (DWI) were also available. All MRI scans were performed after the TFNE. MR images were reviewed blinded to clinical data. Haemorrhagic lesions, ischaemic lesions (chronic or acute) and white matter changes (leukoaraiosis) were recorded according to predefined standardized criteria. The presence and distribution of cerebral microbleeds (CMBs) were evaluated on T2*-GRE images using the Microbleed Anatomical Rating Scale.[238] Prior symptomatic ICH was defined as a symptomatic stroke syndrome associated with imaging evidence of a corresponding ICH (>5 mm in diameter).[198] Asymptomatic prior ICH (>5 mm in diameter) was also noted. Cortical subarachnoid haemorrhage (cSAH) was defined as linear hypointensity in the subarachnoid space affecting one or more cortical sulci of the cerebral convexities on T2*-GRE/SWI sequences with corresponding hyperintensity in the

subarachnoid space on T1-weighted and/or FLAIR images. Cortical superficial siderosis was defined as linear residues of blood products in the superficial layers of the cerebral cortex showing a characteristic "gyriform" pattern of low signal on T2*-GRE images without corresponding hyperintense signal on T1-weighted or FLAIR images. The distribution of superficial siderosis and cSAH was classified as either focal (restricted to ≤3 sulci) or disseminated (≥4 sulci).[195] Leukoaraiosis was assessed with the four-step simplified Fazekas visual rating scale, from 0 to 3 (0=no lesions; 1=focal lesions; 2=early confluent; 3=confluent).[239] After MRI analysis, haemorrhagic lesions (ICH, microbleeds, cSAH and superficial cortical siderosis) or ischaemic lesions (chronic or acute) in the cerebral location corresponding to the clinical features of the TFNE were documented. For patients with a symptomatic ICH following their TFNE, we determined whether the location of the recurrent ICH corresponded to the likely anatomical origin of the preceding TFNE.

2.2.4 Systematic review: Search strategy, selection criteria and data extraction

We undertook a systematic review of all published cases of sporadic CAA with clearly documented TFNE and no known alternative explanation other than CAA. Articles published in full in any language were identified through a search of PubMed and Embase (January 1970 - October 2011) (Appendix A, Table 1). Reference lists from all included articles were also searched for relevant publications. We extracted and used individual patient data from each study where available (including follow-up data on ICH).

2.2.5 Statistical analysis

Survival analysis was used to examine the time to symptomatic ICH from the start of TFNE using Kaplan-Meier analysis. Cox proportional hazard analyses and log rank tests were used to compare the time to symptomatic ICH according to whether patients had experienced predominantly positive or negative symptoms. The proportional hazards assumption was tested, to ensure that there was no evidence of non-proportionality (P>0.2). Multivariable Cox proportional hazards analyses were performed after adjusting for gender and age. Other statistical tests were been used as indicated: continuous variables were compared using Student's t-test (normally distributed) or Mann-Whitney U test (non-normally distributed) and categorical variables using Chi-squared tests or Fisher's exact test.

All statistical tests were two-sided. Analyses were performed using Stata 11.2 (StataCorp LP, Texas).

We prepared this report according to STROBE guidelines for observational studies.[240]

2.3 Results

2.3.1 Multicentre cohort study

In our multicentre cohort study we identified 172 patients with CAA; of these, 25 (definite CAA=1; probable CAA with supporting pathology=2; probable CAA=18; possible CAA=4) (14.5%; 95% CI: 9.6% to 20.7%) had a history of TFNE meeting the study inclusion criteria (Table 2.1).

Thirteen patients (52%) had predominantly positive ("aura-like") symptoms; 12 (48%) had predominantly negative ("TIA-like") symptoms. The most common positive symptom was transient paraesthesias (with or without numbness), in eight patients (32%); a gradual spread to continuous body parts was described in five of these. The sensory symptoms affected the mouth or hand in all cases, and both regions in four cases. Four patients (16%) had limb-jerking episodes and four (16%) patients had episodes of visual disturbances involving monocular blurred vision, flickering or flashing lights, transient "zigzags" (teichopsia), or visual loss. Four (16%) patients had focal weakness and seven (28%) had dysphasia. Most participants (17/25; 68%) had multiple (≥2) stereotyped episodes. The episodes typically lasted less than 10 minutes and in 70% of the patients less than 30 minutes. In seven patients (28%), antiplatelets or anticoagulants were started following TFNE.

All except one patient (with pathologically proven CAA) had a brain MRI undertaken after the TFNE; the median time from the episodes to MRI was 7 days (interquartile range: 6.5-30 days). Nineteen patients (76%) had evidence of lobar ICH: 9 patients (36%) had evidence of acute lobar ICH while 10 patients (40%) had evidence of chronic lobar ICH. Only one patient had a previous cortical infarct. Multiple strictly lobar CMBs were present in 14 patients (58%), cortical superficial siderosis or cSAH in 14 cases (58%). DWI was available in 17 patients (71%) and susceptibility-weighted imaging (SWI) in ten patients (42%). Among patients with DWI, seven (41%) were scanned within 2 weeks of the start of TFNE; acute ischaemia was noted in only one of these patients.

Table 2.1 Demographic, clinical and MRI characteristics of multicentre cohort.

Characteristics		Values (n=25)
Clinical demographics		
Age, mean (95% CI:), years		69.7 (66.9 to 72.5)
Sex, male (%)		14 (56)
Hypertension (%)		13 (52)
Smoking history (%)		3 (13)
On antithrombotics at onset of TFNE(%)		8 (32)
Previous symptomatic ICH (%)		8 (32)
Clinical features of TFNE		
Predominantly positive symptoms (%)		13 (52)
Predominantly negative symptoms ("TIA-like") (%)		17 (68)
Associated headache (%)		3 (12)
Patients with >1 type of attack (%)		4 (16)
Patients with multiple (≥2) episodes (%)		17 (68)
Episodes' duration		
<6 minutes (%)		11 (44)
6-19 minutes (%)		5 (20)
>20 minutes (%)		9 (36)
MRI findings (n=24)		
Median time (days) from episodes to MRI (interquartile range)		7 (6.5-30)
Superficial cortical siderosis (%)		**12 (50)**
Focal (≤3 sulci) (%)		4 (17)
Disseminated (≥4 sulci) (%)		8 (33)
cSAH (%)		4 (17)
cSAH or superficial cortical siderosis (%)		14 (58)
Acute ischaemic lesions		1
Evidence of lobar ICH (%)		19 (76)
Acute lobar ICH evidence (%)		9 (36)
Chronic lobar ICH evidence (%)		10 (40)
Multiple lobar cerebral microbleeds (≥2) (%)		14 (58)
Leukoaraiosis: Fazekas category (%)	0 1 2 3	4 (16) 9 (36) 8 (32) 4 (16)
Antiplatelets/anticoagulants given after TFNE (%)		7 (28)
Aspirin		6 (24)
Aspirin + Clopidogrel		1 (4)
Follow-up information (n=24)		
Median follow-up duration (interquartile range)		14 months (4-35 months)
Occurrence of symptomatic ICH (%)		12 (50)

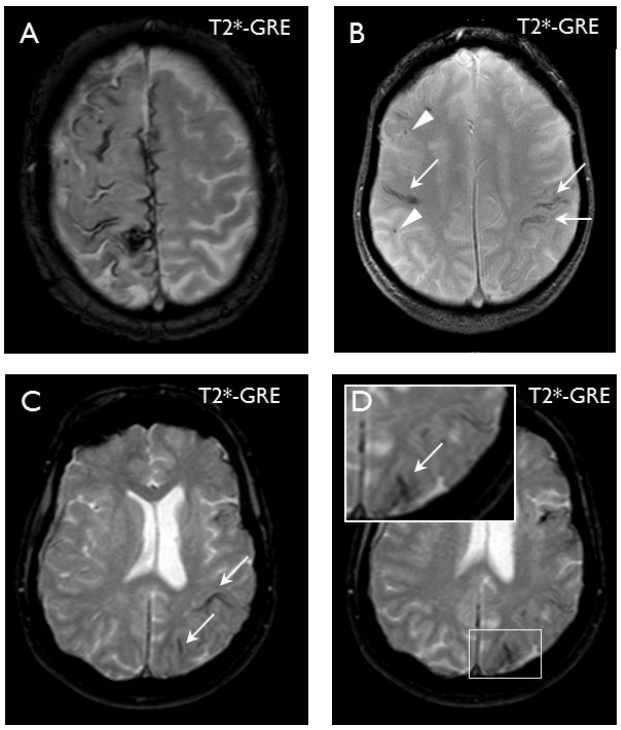

Figure 2.1 (A) Patient 7 had recurrent brief episodes of smoothly migrating tingling, starting in the fingers of the left hand: T2*-GRE MRI shows extensive superficial cortical siderosis in the right hemisphere and lobar cerebral microbleeds (CMBs). (B) Patient 2 had recurrent episodes of tingling in the right thumb, index and middle finger: T2*-GRE shows cortical superficial siderosis (arrows) and lobar CMBs (arrowheads). (C-D) Patient 12 had episodes of visual disturbances in the right hemifield: T2*-GRE shows superficial cortical siderosis in the parietal and occipital lobes.

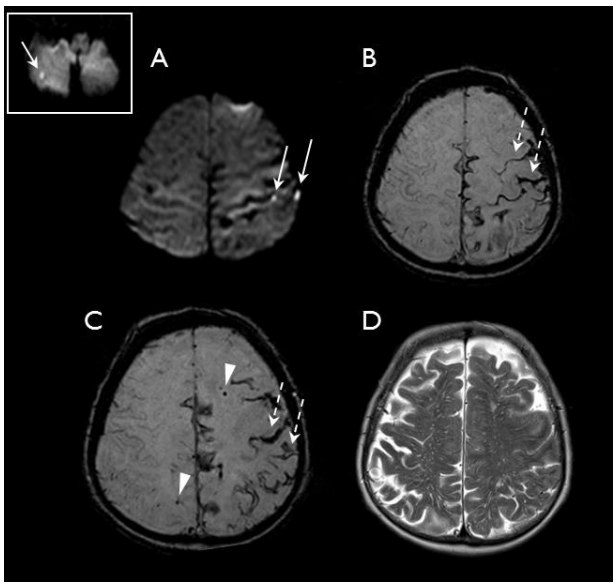

Figure 2.2 Patient 20 experienced two episodes of dysphasia and lightheadedness. (A) Diffusion-weighted imaging one week after the first episode shows two small ischaemic lesions in the left parietal lobe and a lesion in the right cerebellar hemisphere (inset). (B) Susceptibility-weighted imaging indicates cortical superficial siderosis in the sulci very close the ischaemic lesions. (C) Cortical superficial siderosis extends to many sulci, especially in the left hemisphere (dotted arrows), associated with lobar microbleeds (arrowheads) [not visible on T2-weighted MRI (D)].

In 23 patients (92%; patients 1-4, 6-16 and 18-25) the clinical features of the TFNE could be anatomically correlated with haemorrhagic cortical or cortico-subcortical radiological lesions (Figure 2.1and Figure 2.2). Inter-ictal electroencephalography (EEG) was obtained in 5 patients (Patients 1, 8, 13, 17 and 25) and did not show any epileptiform features.

Pathological samples were available in three of the 25 patients (Patient 5, 17 and 18); haematoxylin and eosin staining and immunohistochemical detection of amyloid-β revealed moderate to severe CAA, without vasculitis. In patient 5, who presented with multiple, recurrent episodes of sudden numbness of the right hand, a biopsy of the left temporal lobe revealed multiple cortical micro-infarcts, Alzheimer-type pathology and severe CAA (without vasculitis).

Follow-up data was available in all except one patient with TFNE over a median duration of 14 months (interquartile range 4 to 35 months), during which 12 of 24 patients (50%) had a symptomatic spontaneous lobar ICH; three had multiple consecutive ICHs. Only one patient had an acute ischaemic stroke. For seven patients with ICH occurring after TFNE (58%) the subsequent ICH was in a cortical area corresponding to the likely origin of their TFNE (based on the clinical presentation). Kaplan–Meier ICH analysis indicated that within two months of the TFNE 37.5% (95% CI: 21.6% to 59.7%) of the patients experienced a symptomatic ICH (Figure 2.3). Patients with a subsequent ICH did not differ from those without future ICH, either in clinical and imaging characteristics nor in antiplatelet or anticoagulant use (data not shown).

Patients with and without TFNE were not significantly different in age, prevalence of vascular risk factors, antithrombotic use or previous history of symptomatic lobar ICH (Table 2.2). Among neuroimaging characteristics, the prevalence of superficial cortical siderosis was significantly higher in patients with TFNE compared to those without (50% *vs.* 19%; p= 0.001). Disseminated cortical superficial siderosis (≥4 sulci) was more than twice as common in CAA patients with TFNE compared to those without (33% vs.14%; p=0.005). There were no significant differences in other neuroimaging findings (acute ischaemic lesions, leukoaraiosis, cSAH, presence of multiple lobar CMBs, presence of ICH, or CMB count) between patients with and without TFNE.

2.3.2 Systematic review and meta-analysis

We included 21 studies in the systematic review containing data on 68 patients with CAA-related TFNE (Appendix A, Figure 1). Previously published studies had a significantly higher proportion of "aura-like" positive spreading sensory disturbances compared to our multicentre cohort (82% vs. 36%; p<0.0001) (Figure 2.4). Fifteen studies (six case series and nine case reports; n=43) had adequate follow-up data. This population was similar in age, gender and proportion of cases with a history of prior symptomatic ICH to our multicentre cohort.

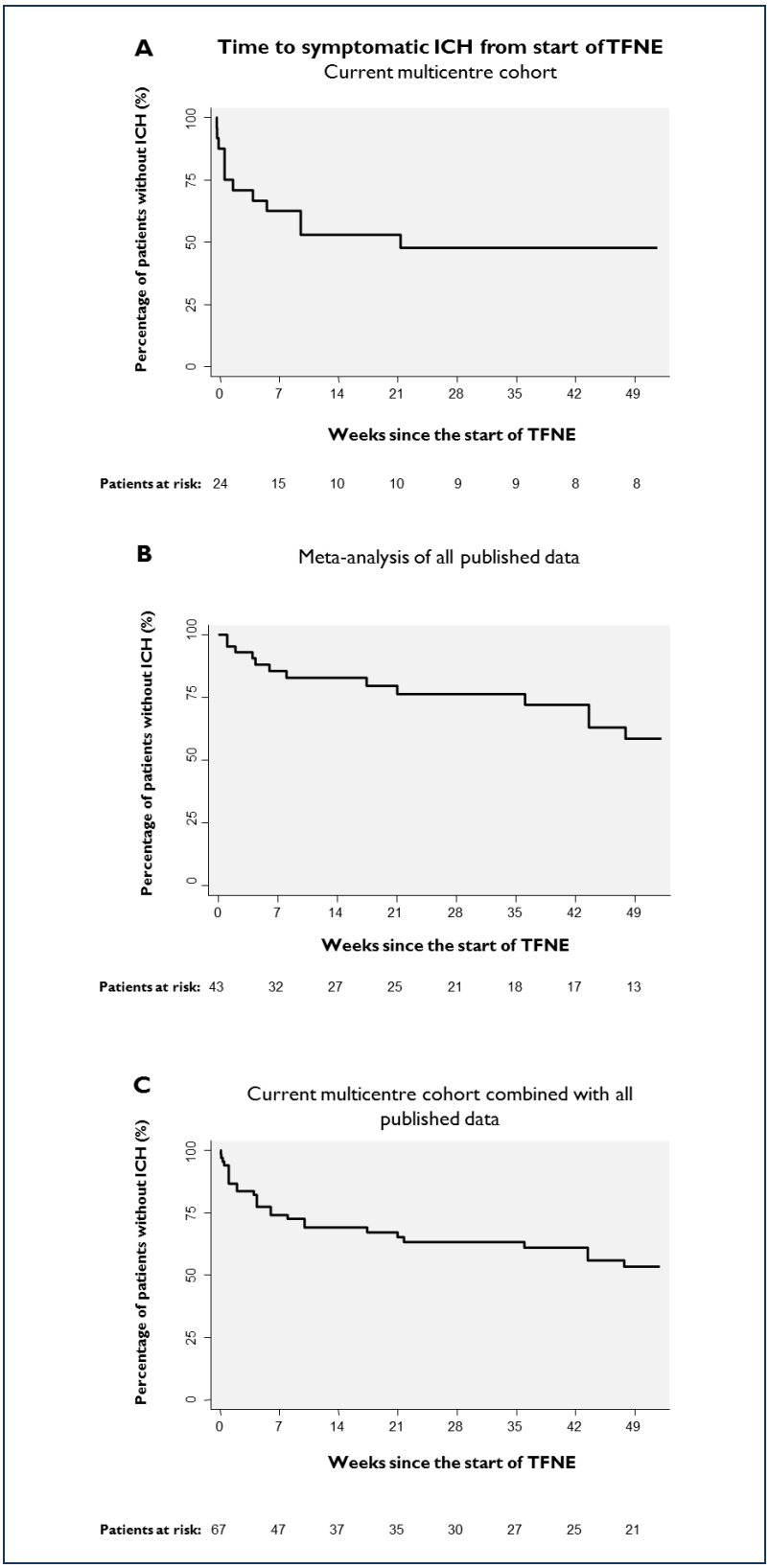

Figure 2.3 Kaplan-Meier analyses for event-free rates of symptomatic intracerebral haemorrhage after transient focal neurological episodes (TFNE) in our multicentre cohort (n=24; A), all published studies (n=43, B), and combined (n=67; C).

Table 2.2 Characteristics of patients with cerebral amyloid angiopathy (CAA) and transient focal neurological episodes (TFNE) vs. CAA patients without TFNE.

Characteristics	CAA patients with TFNE (n=25)	CAA patients without TFNE (n=147)	P-value
Age, mean (95% CI:), years	69.7 (66.9-72.5)	73.1 (71.4-74.9)	0.08
Sex, male (%)	14 (56)	73 (50)	0.558
Hypertension (%)	13 (52)	92 (67)	0.145
On antithrombotics (%)	8 (32)	41 (30)	0.801
Previous symptomatic ICH (%)	8 (32)	54 (39)	0.532
Superficial cortical siderosis (%)	12 (50)	24 (19)	0.001
Focal (≤3 sulci) (%)	4 (17)	10 (8)	0.187
Disseminated (≥4 sulci) (%)	8 (33)	14 (11)	0.005
cSAH (%)	4 (17)	8 (7)	0.106
cSAH or superficial cortical siderosis (%)	14 (58)	30 (25)	0.001
Acute ischaemic lesions (%)	1 (6)	12 (11)	0.512
Chronic lobar ICH evidence (%)	10 (40)	72 (53)	0.248
Acute lobar ICH evidence (%)	9 (36)	65 (48)	0.250
Multiple lobar CMBs (≥2) (%)	14 (58)	71 (56)	0.975
Number of CMBs, median (IQR range)	3 (0-16)	2.5 (0-8)	0.552
Leukoaraiosis: Fazekas category (%)			
0	4 (16)	25 (19)	0.981
1	9 (36)	43 (32)	
2	8 (32)	43 (32)	
3	4 (16)	22 (17)	

A meta-analysis combining these 15 studies with our cohort showed a future risk of symptomatic ICH of 27.6% (95% CI: 18.3% to 40.2%) at two months (Figure 2.3). Patients with negative symptoms had a similar risk of future ICH to patients with positive symptoms (hazard ratio: 0.91; 95% CI: 0.40-2.08; p=0.83). There was a borderline significant lower risk of future ICH among patients with prior symptomatic ICH compared to those without (hazard ratio: 0.47; 95% CI: 0.22-1.01).

2.4 Discussion

In our multicentre cohort study we found TFNE in 14% of patients with CAA. Our study confirms previous reports that CAA-related TFNE are mostly recurrent, stereotyped and brief (usually >30 minutes).[167, 168] However, unlike published studies, in which "aura-like" sensory episodes seem to be most frequent, in our cohort, negative symptoms were just as common. This difference might reflect previous publication bias in favour of CAA cases

with spreading sensory phenomena not typical of TIAs. All episodes of aura-like sensory symptoms in our cohort (n=8) involved the face or hand; three involved the corner of the mouth and the hand consistent with a cortical cheiro-oral syndrome. In two of these, cortical siderosis/cSAH were present over the frontal or parietal lobes, whilst the third case had multiple lobar CMBs. Although the numbers of cases was small, the cheiro-oral syndrome may be rather characteristic of CAA-related TFNE.

The clinical features of the episodes indicate a cortical rather than a subcortical origin; moreover, they were often correlated anatomically with haemorrhagic MRI lesions, including CMBs, superficial cortical siderosis and lobar ICH. Thus, TFNE are probably related to the haemorrhagic rather than the ischaemic components of CAA: possible mechanisms include focal seizure-like activity or migraine aura-like cortical spreading depression, as suggested by previously published small case series.[167, 168, 241] Twenty three patients in our cohort had haemorrhagic imaging findings in a neuroanatomical location corresponding to their TFNE symptoms; eight had superficial cortical siderosis, reflecting previous episodes of acute bleeding in the subarachnoid space of adjacent cortical sulci. Three other recent case series have also emphasized the possible role of non-traumatic cSAH in CAA-related TFNE.[171, 191, 194] Our finding that superficial cortical siderosis was significantly more common in CAA patients with TFNE than those without (p=0.001) provides strong evidence that this pattern of bleeding is likely to be an important cause of TFNE.

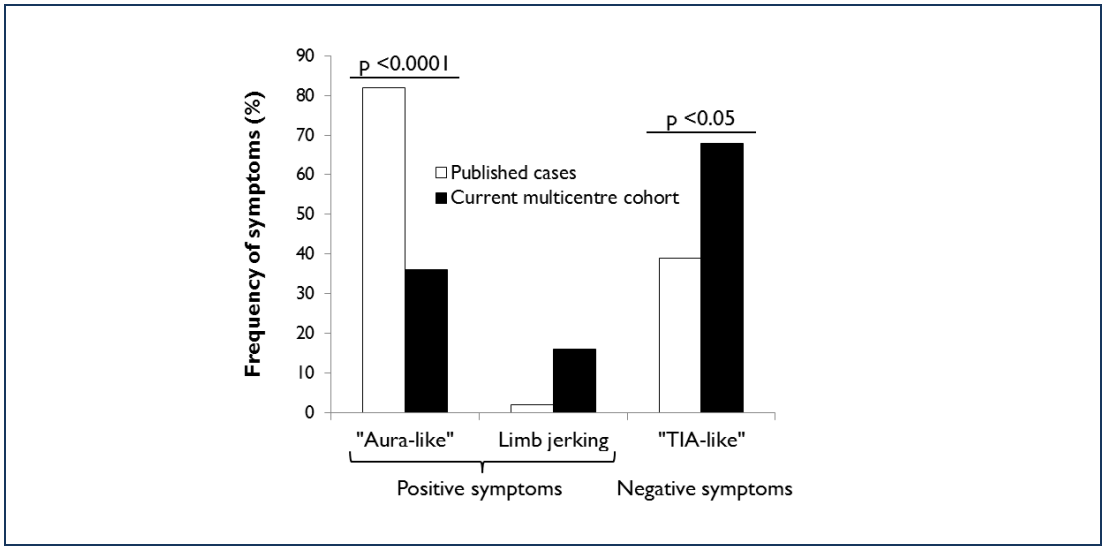

Figure 2.4 The prevalence of different types of transient focal neurological symptoms in published cases (n=54) and in our multicentre cohort of CAA patients. Percentages were compared using Pearson Chi-squared tests. (Percentages do not add up to 100 as some patients experienced multiple episode types).

Nevertheless, a role for ischaemic lesions in CAA-related TFNE cannot be ruled out by our study: small (apparently asymptomatic) ischaemic lesions have been detected *in vivo* in clinically probable CAA,[73] and "microinfarcts" are a frequent neuropathological finding in the brains of patients with CAA.[28] Since not all cases in our study had DWI soon after the onset of TFNE, we may have underestimated the contribution from small acute ischaemic lesions.

We report a strikingly high early risk of symptomatic lobar ICH (37.5% at two months) following CAA-related TFNE, which we confirmed in a meta-analysis of our data with all published studies. We found only a borderline difference in the risk of ICH among patients with previous symptomatic ICH compared to those without, suggesting that TFNE, rather than simply the presence of CAA with previous symptomatic ICH (with a known recurrent ICH risk of up to about 10% per year[28, 37]), are an independent marker of high early future ICH risk. TFNE in CAA may thus be a clinical marker for cerebral areas of focally-active and severe CAA pathology, with more vascular leakage leading to an increased risk of future ICH. This is supported by our finding of a significantly higher prevalence of superficial cortical siderosis in CAA patients with TFNE, since superficial siderosis is hypothesized to result from recurrent bleeding into the subarachnoid space, leading to subpial accumulation of blood-breakdown products. However, the role of superficial siderosis as a prognostic imaging marker of increased ICH risk in CAA requires further study.

Although in 58% of patients with ICH following TFNE the new haematoma was located in a cortical area corresponding to the likely origin of their TFNE (based on the clinical presentation), further work is also needed to establish whether this apparent clustering of ICH in brain regions implicated by TFNE symptoms is greater than predicted by chance.

Our study has several potential limitations. We may have underestimated the true prevalence of CAA-related TFNE because of the retrospective study design; further large prospective studies with systematic enquiry about previous TFNE are needed. Some of our CAA cohort may have been misdiagnosed due to the imperfect specificity of the Boston criteria (particularly the "possible CAA" category).[202] MRI was performed at different times from TFNE onset; this, combined with the lack of availability of acute DWI sequences in all cases may have influenced the detection of haemorrhagic over ischaemic lesions. We also acknowledge the potential for referral, selection and publication bias in the cases identified in the systematic review. Our multicentre cohort results might not be generalizable to all CAA patients, but only to those presenting to vascular neurology services in whom other

possible causes of transient focal neurological symptoms have been excluded. Although this study includes the largest number of CAA-related TFNE cases to date, we did not have sufficient statistical power to definitively determine potential predictors for ICH.

We have shown that TFNE are common in CAA and signify a very high early future risk of ICH. Hence, their diagnosis has important clinical implications. Our findings clearly suggest a key role for T2*-GRE MRI (or other blood-sensitive sequences) in the investigation of patients with unexplained TFNE-especially in individuals without known risk factors for TIA. The very high early risk of lobar ICH following TFNE in CAA may be an opportunity to commence preventive strategies: we suggest clinicians should discontinue and avoid giving antiplatelets or anticoagulants in cases of TFNE with imaging evidence of CAA, even if the episodes seem clinically likely to be ischaemic. Since TFNE were observed in patients without a previous history of symptomatic ICH, they may also prove to be a useful diagnostic marker of CAA, potentially allowing diagnosis earlier in its disease course.[28]

Chapter 3 Prevalence and mechanisms of cortical superficial siderosis in sporadic cerebral amyloid angiopathy

ABSTRACT

Objective: We investigated the prevalence and clinical-radiological associations of cortical superficial siderosis (cSS) in patients with probable cerebral amyloid angiopathy (CAA) compared to those with intracerebral haemorrhage (ICH) not attributed to CAA.

Methods: Retrospective multicentre cohort study of 120 patients with probable CAA and two comparison groups: 67 patients with either single lobar ICH or mixed (deep and lobar) haemorrhages; and 22 patients with strictly deep haemorrhages. We rated cSS, ICH, white matter changes (WMC) and cerebral microbleeds (CMBs).

Results: cSS was detected in 48/120 (40%; 95%CI: 31.2%-49.3%) patients with probable CAA, 10/67 (14.9%; 95%CI: 7.4-25.7%) with single lobar ICH or mixed haemorrhages, and 1/22 (4.6%; 95%CI: 0.1%-22.8%) patients with strictly deep haemorrhages ($p<0.001$ for trend). Disseminated cSS was present in 29/120 (24%: 95%CI: 16.8-32.8%) probable CAA patients, but none of the other ICH patients ($p<0.001$). In probable CAA, age (OR: 1.09; 95%CI: 1.03-1.15; $p=0.002$), chronic lobar ICH (OR: 3.94, 95%CI: 1.54-10.08; $p=0.004$) and transient focal neurological episodes (OR: 11.08; 95%CI: 3.49-35.19; $p<0.001$) were independently associated with cSS. However, cSS occurred in 17 of 48 probable CAA patients (35.4%, 95%CI: 22.2-50.5%) without chronic ICH.

Conclusions: cSS (particularly if disseminated) is a common and characteristic feature of CAA. Chronic lobar ICH is an independent risk factor for cSS, but the causal direction and mechanism of association is uncertain. Haemorrhage into the subarachnoid space, independent of previous (chronic) lobar ICH, must also contribute to cSS in CAA. Transient focal neurological episodes are the strongest clinical marker of cSS.

3.1 Introduction

Sporadic cerebral amyloid angiopathy (CAA) is a common age-related small vessel disease due to progressive deposition of amyloid–β in the walls of small arteries, arterioles and capillaries in the cerebral cortex and overlying leptomeninges.[28] CAA is most often recognised in life by symptomatic, lobar intracerebral haemorrhage (ICH) in elderly patients.[28, 37, 242] CAA is also associated with characteristic magnetic resonance imaging (MRI) findings including lobar cerebral microbleeds (CMBs) and white matter hyperintensities (leukoaraiosis).[28, 35]

Recent studies have identified cortical superficial siderosis (cSS) as another manifestation of CAA.[171, 191, 194, 195] In CAA, cSS has a characteristic predilection for the cerebral convexities, reflecting linear blood residues in the superficial layers of the cerebral cortex or in the subarachnoid space.[190, 195, 243] cSS may have clinical relevance as an important cause of transient focal neurological episodes (sometimes called "amyloid spells"),[244, 245] and potential "warning sign" for future symptomatic ICH.[246]

Although cSS is a promising diagnostic neuroimaging marker of CAA,[8] the strength of the association and underlying mechanisms have not been systematically studied. In the present study we therefore sought to determine the prevalence and extent of cSS in a European multicentre cohort of patients with clinical-radiological probable CAA, and investigate its associations with other imaging findings including CMBs, white matter changes and ICH. We hypothesized that cSS: (1) is common in subjects with probable CAA, and more prevalent compared to comparison subjects with other spontaneous ICH not attributed to probable CAA; and (2) is associated with other hemorrhagic manifestations of CAA (lobar ICH and lobar CMBs).

3.2 Methods

3.2.1 Study population

We included consecutive patients diagnosed with probable CAA (according to the original Boston criteria[202] – i.e. not including cSS as a criterion, see below) at four stroke centres over defined time periods (Appendix B Figure 1). At participating centres, MRI scanning is a routine investigation for cases of suspected CAA, unless there are contra-indications. Essential inclusion criteria for the main CAA case group were: (1) probable or pathologically verified CAA, defined according to the Boston criteria[202] – including lobar

CMBs, but not cSS; and (2) available MRI sequences of adequate quality including T2*-weighted gradient-recalled echo (T2*-GRE) and FLAIR MRI sequences. Cases defined as possible CAA according to the Boston criteria[202] (i.e. single lobar ICH without lobar microbleeds), were included in one of the comparison groups (see next paragraph).

As comparison groups we included patients with other symptomatic, spontaneous ICH not fulfilling the Boston criteria for probable CAA, seen in the four stroke centres over the same time period, with available MRI scans of adequate quality including T2*-GRE and FLAIR sequences. Cases were systematically and consecutively ascertained using multiple overlapping methods from prospective clinical and radiological databases. The two non-CAA comparison groups were as follows: (a) single lobar ICH (without any CMBs) and mixed (deep and lobar) haemorrhages (including any combination of ICH or CMBs) which we subsequently refer to as "single lobar ICH and mixed haemorrhages"; and (b) strictly deep haemorrhages (including ICH and CMBs in the basal ganglia and brainstem).

In total, 149 subjects were excluded from our study because MRI scans were unavailable (Appendix B Figure 1); those excluded were not significantly different from those included in gender, age or measures of stroke severity (all p values>0.10).

3.2.2 Standard Protocol Approvals, Registrations, and Patient Consents

The study received ethical approval by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee, the Commission d'Ethique Biomedicale Hospitalo Facultaire of the Faculte de Medicine (Universite Catholique de Louvain), and the Comite d'ethique medicale of the CHU Mont Godinne UCL.

3.2.3 Data collection

Demographic and clinical information was obtained from prospective databases and by medical records review using standardised data collection forms. Hypertension was defined as a history of hypertension, taking antihypertensive treatment or documented elevated blood pressure (systolic >150 or diastolic >95mmHg) before admission, diabetes as ongoing use of a hypoglycaemic agent and smoking as history of tobacco use before admission. In patients with probable CAA, a clearly documented history of transient (≤24 hours), fully resolving, focal neurological episodes with no known alternative explanation other than

CAA (e.g. structural brain lesion, atrial fibrillation, extracranial or intracranial stenosis) was ascertained by review of medical records.

3.2.4 Magnetic resonance imaging acquisition and analysis

The MRI protocol was similar in each hospital. Imaging was at 1.5T field strength for all patients and included T1-weighted, T2-weighted, fluid attenuated inversion recovery (FLAIR), T2*-GRE (slice thickness 5 mm, repetition time 500–1,000 ms, echo time 40/26/15/50-70 ms) and diffusion-weighted imaging (DWI) sequences. Images were reviewed blinded to clinical data. Hemorrhagic lesions,[35, 195, 198, 238] ischaemic lesions (chronic or acute)[73] and white matter hyperintensities (leukoaraiosis)[239] were recorded according to predefined standardized criteria. CMBs were evaluated on T2*-GRE images.[35, 238] Prior symptomatic ICH was defined as a symptomatic stroke syndrome associated with imaging evidence of a corresponding "macro" ICH (>5 mm in diameter on T2*-GRE).[198] Asymptomatic prior ICH (>5 mm in diameter on T2*-GRE MRI) was also noted: chronic ICH was defined on neuroimaging as ICH with no acute bleeding identified on either CT or MRI scans. Acute convexity subarachnoid haemorrhage (cSAH) was defined as linear hypointensity in the subarachnoid space affecting one or more cortical sulci of the cerebral convexities on T2*-GRE sequences with corresponding hyperintensity in the subarachnoid space on T1-weighted or FLAIR images. cSS was defined as linear residues of chronic blood products in the superficial layers of the cerebral cortex showing a characteristic "gyriform" pattern of low signal on T2*-GRE images, without corresponding hyperintense signal on T1-weighted or FLAIR images. We did not include cSS if it was contiguous with any ICH. The distribution of cSS and acute cSAH was classified as focal (restricted to ≤3 sulci) or disseminated (≥4 sulci).[195] We also noted whether cSS was contralateral or ipsilateral to any chronic ICH. Using a sample of MRI scans from probable CAA patients (n=48) and control non-CAA cases (n=27), the inter-rater agreement for the presence or absent of cSS was 93.3% (Cohen's kappa=0.85) and for cSS categories was 95.1% (weighted Cohen's kappa=0.86). White matter hyperintensities (leukoaraiosis) were assessed with the four-step simplified Fazekas rating scale, from 0 to 3 (0=no lesions; 1=focal lesions; 2=early confluent; 3=confluent).[239]

3.2.5 Statistical analysis

We compared demographic, clinical and imaging characteristics of probable CAA patients with *versus* without cSS. Both binary and ordinal logistic regression analysis was used to identify predictors of cSS (cSS presence and cSS extent respectively). Multivariable analysis

was adjusted for age, lobar CMBs (used as a categorical variable: 0, 1, 2-4, and >4 CMBs), presence of chronic lobar ICH and history of transient focal neurological episodes, based on the results of univariable analysis, plus other biologically plausible confounders. As a sensitivity analysis we repeated these analyses with the addition of hypertension in our multivariable regression models. Because the results of binary and ordinal logistic regression analysis were consistent, we present only the results of binary logistic regression analysis. A P-value ≤0.05 was considered to be statistically significant. Data was missing in less than 5% of participants; these cases were excluded from univariable and multivariable analyses that included a missing variable of interest. For the main multivariable model presented, there were no missing data. All statistical analyses were carried out using STATA (Version 11.2, StataCorp.).

3.3 Results

The final cohort consisted of 209 patients: 120 patients with probable CAA (9 with supportive pathology) based on the Boston criteria and 89 patients with other ICH not fulfilling the Boston criteria for probable CAA, forming two comparison groups: 67 patients with a single lobar ICH or mixed (lobar and deep) haemorrhages; and 22 patients with strictly deep haemorrhages (Table 3.1).

Table 3.1 Characteristics of patients with probable cerebral amyloid angiopathy (CAA) and our comparison patients groups with spontaneous symptomatic ICH not fulfilling the Boston criteria for probable CAA: "single lobar ICH and mixed haemorrhages" and "strictly deep haemorrhages".

Characteristics	Probable CAA (n=120)	Comparison groups not fulfilling criteria for probable CAA	
		Single lobar ICH/mixed deep and lobar haemorrhages (n=67)	Strictly deep haemorrhages (n=22)
Age, mean (95% CI:), years	71.8 (70-73.5)	72.6 (61.3-75.3)	61.2 (58.5-71.8)
Sex, male (%)	67 (55.8)	35 (52.3)	15 (68.2)
Hypertension (%)	72 (64.3)	48 (71.6)	21 (99.5)
On antithrombotics (%)	25 (22.1)	24 (35.8)	7 (31.8)
Any symptomatic ICH (%)	104 (86.7)*	67 (100)	22 (100)
cSS (%)	48 (40)	10 (14.9)	1 (4.6)
Focal, ≤3 sulci (%)	19 (15.8)	10 (14.9)	1 (4.6)
Disseminated, ≥4 sulci (%)	29 (24.2)	0 (0)	0 (0)
Acute cSAH (%)	15 (12.5)	3 (4)	0 (0)

* One hundred-four of the probable CAA patients were admitted for spontaneous lobar ICH, 8 with transient focal neurological episodes, 2 due to cognitive decline, 2 with acute convexity subarachnoid haemorrhage and 2 because of an ischaemic stroke.

cSS was detected in 48 out of 120 (40%; 95%CI: 31.2%-49.3%) patients with probable CAA, compared to 10/67 (14.9%; 95%CI: 7.4-25.7%) of the patients with a single lobar ICH or mixed (lobar and deep) haemorrhages, and only 1/22 (4.6%) patients with strictly deep haemorrhages (p<0.001 for trend) (Table 3.1). Within the single lobar ICH or mixed haemorrhage group, cSS was found in 2/33 (6.1%) of patients with single lobar ICH and no CMBs, 2/11 (18%) of patients with lobar ICH and deep or mixed CMBs, and 3/17 (18%) of patients with deep ICH and lobar or mixed CMBs (see Appendix B Table 1 for the frequency of cSS in detailed patient subgroups). Disseminated cSS was observed in 29/120 of probable CAA patients (24.2%; 95% CI 16.8-32.8) but in none of the other 89 patients not fulfilling the original Boston criteria for probable CAA (p<0.001). In patients with single lobar ICH or mixed haemorrhages, cSS (10/67) was always focal; of these 10 patients with cSS, 7 (70%) had lobar CMBs (Appendix B Table 2).

Table 3.2 Characteristics and comparison of CAA patients with and without superficial cortical siderosis. P-values refer to differences between CAA patients with vs. without cortical superficial siderosis (cSS), using chi-square tests and the Fisher's exact test for categorical variables, and two-sample t-tests or Mann-Whitney U-tests depending on the distribution of continuous variables.

Characteristics	All probable CAA (n=120)	cSS (+) (n=48)	cSS (-) (n=72)	p-value
Age, mean (95% CI:), years	71.8 (70-73.5)	74.8 (72.3-77.3)	69.7 (67.4-72.1)	0.005
Sex, male (%)	67 (55.8)	25 (52.1)	42 (58.3)	0.499
Hypertension (%)*	72 (64.3)	20 (46.5)	52 (75.4)	0.002
On antithrombotics (%)*	25 (22.1)	9 (20.9)	16 (22.9)	0.811
History of prior ICH (%)*	46 (40.7)	18 (41.9)	28 (40)	0.845
History of TFNE (%)	26 (21.7)	19 (39.6)	7 (9.7)	<0.001
Chronic lobar ICH (%)	61 (51.3)	31 (64.6)	30 (42.3.)	0.017
Acute lobar ICH (%)	57 (48.3)	26 (54.2)	31 (44.3)	0.291
Acute ischaemic lesions (%)	20 (18.7)	10 (23.3)	10 (15.6)	0.321
Number of CMBs, median (IQR range)	4 (2-13.5)	4.5 (1-10.5)	4 (2-15.5)	0.147
Lobar CMBs				
0 (%)	13 (10.8)	9 (18.8)	4 (5.6)	
1 (%)	16 (13.3)	7 (14.6)	9 (12.5)	
2-4 (%)	34 (28.3)	8 (16.7)	26 (36.1)	
≥5 (%)	57 (47.5)	24 (50)	33 (45.8)	
Acute cSAH (%)	15 (12.5)	9 (18.8)	6 (8.3)	0.091
Moderate-severe leukoaraiosis (%)	63 (53.4)	22 (46.8)	41 (57.6)	0.244

† p-value for trend; *There were 10 patients with missing data for one or more of these variables: Hypertension (n=9), On antithrombotics (n=8), History of prior symptomatic ICH (n=7).

Thirty-one of the 48 probable CAA patients with cSS had chronic lobar ICH; the cSS was contralateral to the ICH or bilateral in 24 of these patients (87.4%) and restricted to the hemisphere ipsilateral to chronic lobar ICH in only 7 patients (22.6%) (i.e. in most cases, cSS was present distant from any chronic lobar ICH). In 17 of the 48 probable CAA patients with cSS (35%) there was no evidence of chronic lobar ICH. Representative examples of cSS are shown in Figure 3.1.

Within the probable CAA group, patients with cSS were older and less often hypertensive compared to patients without cSS (Table 3.2). Patients with cSS more often had a history of transient focal neurological episodes and chronic lobar ICH on MRI than patients without cSS. However, we found no association between the presence of cSS and acute lobar ICH, lobar CMBs or white matter hyperintensity severity.

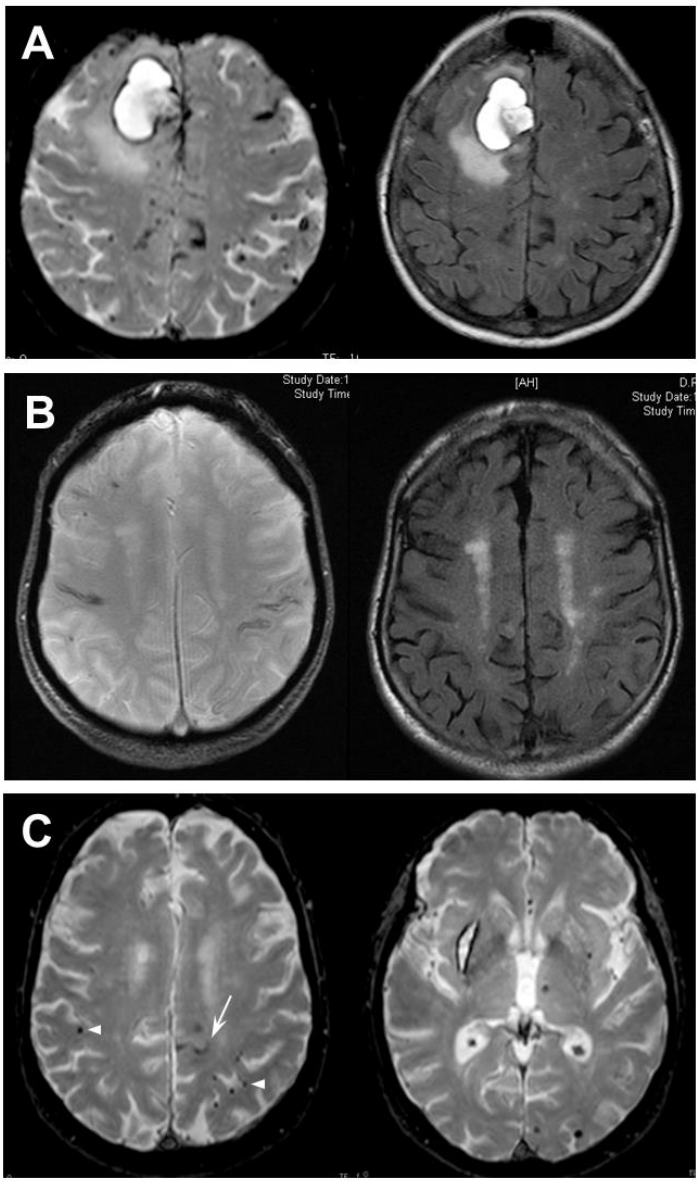

Figure 3.1 T2*-GRE and FLAIR MRI of two patients with probable cerebral amyloid angiopathy and a patient with mixed deep and lobar haemorrhages. (A) A patient with probable CAA and an acute right frontal intracerebral haemorrhage (ICH) and multifocal cortical superficial siderosis (cSS) in the left hemisphere. Multiple lobar cerebral microbleeds (CMBs) are also present, sometimes close to cSS. (B) A patient with probable CAA with focal, bilateral cSS and lobar CMBs, in whom no ICHs were present. (C) A patient with a mixed pattern of bleeds (ICH and CMBs) not fulfilling the Boston criteria for CAA: note the focal cSS in left occipital lobe and the lobar CMBs in close vicinity to cSS.

Table 3.3 Multivariate regression analysis showing the factors associated with cortical superficial siderosis in patients with probable cerebral amyloid angiopathy. The model remains consistent when history of hypertension and acute cSAH (%) are included.

	OR (95%CI)	P-Value
Age (years)	1.09 (1.03-1.15)	0.002
Lobar CMBs (for each category increase)	0.77 (0.50-1.21)	0.260
Chronic lobar ICH	3.94 (1.54-10.08)	0.004
History of transient focal neurological episodes	11.08 (3.49-35.19)	<0.001

In univariable logistic regression analysis, factors associated with cSS were age, history of transient focal neurological episodes and chronic lobar ICH (Appendix B Table 3). Hypertension showed a negative association with cSS. In multivariable logistic regression analysis, age, presence of chronic lobar ICH and history of transient focal neurological episodes (were independently associated with cSS, after adjusting for lobar CMBs (Table 3.3). These results remained consistent after additional adjustment for hypertension and acute cSAH (data not shown). The results of cSS predictors in ordinal logistic regression (i.e. predictors of cSS extent: no cSS vs. focal vs. disseminated) were consistent and of similar effect size.

3.4 Discussion

To our knowledge, this multicentre retrospective study is the first systematic survey of the prevalence and clinical-radiological associations of cSS in patients with probable sporadic CAA (diagnosed by the original Boston criteria) compared to those with ICH not fulfilling these criteria. A previous study investigated the presence of cSS in patients diagnosed with CAA on histopathology:[195] cSS was detected in 60.5% of patients with histopathologically-proven CAA (n=38; mean age 70 years), compared with none of the controls with histopathologically proven non-CAA ICH (n=22; mean age 54 years).[195] The authors suggested that cSS might be helpful for the clinical diagnosis of CAA.[195] cSS has also been found in patients with hereditary cerebral haemorrhage with amyloidosis–Dutch type, a distinct genetic type of CAA, and always in the direct vicinity of a lobar ICH or a CMB.[203] Our findings strengthen the hypothesis that cSS (especially if disseminated) is a characteristic neuroimaging marker for CAA. We noted cSS in 40% of probable CAA patients, disseminated cSS only in patients with probable CAA, and found that cSS is much rarer (prevalence less than 5%) in patients with a "strictly deep" pattern of ICH than in

probable CAA. We also detected cSS in 15% of patients with a single lobar ICH or mixed (lobar and deep) haemorrhages; however, in most of these mixed patients, lobar CMBs were also present, suggesting that they might in fact harbour some degree of CAA pathology.

Among patients with a single lobar ICH and no CMBs (i.e. "possible CAA" according to the original Boston criteria[202]) the prevalence of cSS was also very low (6%). In a validation of these criteria, 16 of 26 patients (62%) classified as possible CAA had pathologically-confirmed CAA,[202] but only 11 of the pathologically diagnosed patients had T2*-GRE imaging, limiting the generalizability of the findings to current cohorts (including our study), in which such imaging is now routine. In another validation study of the Boston criteria, using T2*-weighted gradient echo MRI in a hereditary Dutch-type CAA population, all patients with lobar ICH also had CMBs.[203] Our data suggest that further pathological correlation is required to determine the prevalence of pathologically-confirmed CAA in patients with a single lobar ICH (and no CMBs or cSS) on appropriate blood-sensitive MRI sequences. Further studies with histopathological confirmation or *in vivo* amyloid imaging[176] will also help to determine whether cSS is a useful diagnostic feature of CAA in patients with mixed patterns of haemorrhage.

In the healthy population-based Rotterdam Scan Study cSS was found in 0.7% (7/1062) of elderly individuals (mean age 69.6 years), all of whom had lobar CMBs (6 had strictly lobar CMBs, 5 of whom had multiple CMBs), in close vicinity to the cSS.[243] In agreement with our findings, the mean age of persons with cSS was higher than those without cSS (mean age 79.9 vs. 69.6 years; $p<0.001$), and also compared to persons who had CMBs but no cSS (mean age 71.8 years).[243] Since the severity of CAA is age-related,[28, 37] these finding, together with our data showing an association of cSS with chronic ICH, suggest that cSS might be a marker of more advanced CAA, but this also requires pathological confirmation, with standardized grading of CAA severity.

Experimental studies confirm that repeated bleeding into the subarachnoid space leads to subpial haemosiderin deposition.[247] There are thus at least two possible pathophysiological mechanisms which could lead to cSS deposition in CAA: (a) repeated episodes of haemorrhage from brittle superficial cortical or leptomeningeal CAA-affected vessels into the subarachnoid space (independent of lobar ICH); and (b) leakage from a previous lobar ICH (or superficial lobar CMBs) into the subarachnoid space. Our observation of cSS in 17 patients without chronic lobar ICH, and even in those with chronic lobar ICH mostly distant from the ICH (in nearly 90% of cases in the contralateral hemisphere or bilaterally), favours a contribution from direct haemorrhage into the

subarachnoid space, independent of lobar ICH. This implies that cSS may arise independently of known characteristic imaging features of CAA (lobar ICH and CMBs), supporting its role as an independent diagnostic marker; further studies are needed to confirm the value of cSS in improving the sensitivity of in vivo CAA diagnosis.

Perhaps surprisingly, we did not find any association between lobar CMBs and cSS, which may reflect selection bias towards generally advanced disease with high lobar CMBs prevalence in our cohort. Nevertheless in some cases cSS was observed close to one or more lobar CMBs (see example in Figure 2), suggesting that leakage from very superficial CMBs may also be a mechanism of cSS.

Another interpretation of the association between cSS and lobar ICH is that cSS *precedes* lobar ICH. Although our cross-sectional data cannot confirm this hypothesis, some recent data support this explanation. A retrospective study of 51 patients with cSS and due to possible or probable CAA found that after a median 35.3 months follow-up, 47.1% of the patients had new radiological ICH or cSAH, often at the site of pre-existing siderosis,[246] providing preliminary evidence that cSS heralds a high risk of future ICH. A small neuropathological series of six autopsy cases of subcortical haematoma caused by CAA showed that at least in some cases the primary haemorrhage appeared to originate from the subarachnoid space.[248] Further prospective studies are urgently required to determine the risk of future intracranial bleeding associated with cSS in CAA.

Our study confirms a strong independent association of cSS with transient focal neurological episodes (sometimes called "amyloid spells"[249]), which are increasingly recognized in CAA and can resemble transient ischaemic attacks, migraine auras or focal seizures.[167, 244, 249] Such attacks could plausibly be caused by disruption of cortical function due to cSS, for example by superficial cortical haemosiderin deposition inducing focal seizures or cortical spreading depression.[244, 249-251] CAA-related transient focal neurological episodes are associated with a high early risk of symptomatic lobar ICH (24.5% [95% CI: 15.8%–36.9%] at 8 weeks);[244] cSS may be one mechanism underpinning this increased clinical risk, reflecting focally active CAA near the cortical surface.[252]

Our study has several strengths including the systematic evaluation of MRI scans by trained raters using validated scales for a range of imaging markers of small vessel disease. A limitation is the lack of pathological confirmation of the CAA pathology. In view of the imperfect specificity of the Boston criteria, especially for the "possible CAA" category,[202] we focussed on patients fulfilling the criteria for probable CAA, for which the specificity is between 82-100%.[202, 203] Limitations of our study include the retrospective design, the variation in inception point of the disease at inclusion, and the potential of bias in our

sample as MRIs were done as part of routine clinical care, tending to exclude more severe cases of CAA and ICH.

Our study indicates that cSS (particularly if disseminated) is a characteristic neuroimaging feature of CAA. Chronic lobar ICH is an independent risk factor for cSS, but the causal direction and mechanism of the association is uncertain: although leakage from previous lobar ICH into the subarachnoid space may lead to cSS, it is also possible that cSS heralds an increased risk of future lobar ICH. Further prospective studies are needed to clarify how cSS relates to future ICH risk, which may have important clinical relevance, for example regarding antithrombotic treatment. Our results also show that haemorrhage into the subarachnoid space, independent of lobar ICH, must also contribute to cSS in CAA, suggesting that cSS should be considered an additional neuroimaging marker of CAA-related haemorrhage.

Chapter 4 Cortical superficial siderosis and intracerebral haemorrhage risk in patients with sporadic cerebral amyloid angiopathy: multicentre MRI cohort study

ABSTRACT

Objective: To investigate whether cortical superficial siderosis (cSS) on MRI, especially if disseminated (involving more than 3 sulci), increases the risk of future symptomatic lobar intracerebral haemorrhage (ICH) in cerebral amyloid angiopathy (CAA).

Methods: European multicentre cohort study of 118 CAA patients (104 with baseline symptomatic lobar ICH) diagnosed according to the Boston criteria. We obtained baseline clinical, MRI and follow-up data on symptomatic lobar ICH. Using Kaplan-Meier and Cox regression analyses, we investigated cSS and ICH risk, adjusting for known confounders.

Results: During a median follow-up time of 24 months (interquartile range 9–44 months), 23/118 (19.5%, 95%CI: 12.8-27.8) patients experienced symptomatic lobar ICH. Any cSS, and disseminated cSS were predictors of time until first or recurrent ICH (log-rank test: p=0.0045 and p=0.0009 respectively). ICH risk was 25% (95%CI: 7.6-28.3%) for patients without siderosis; 28.9% (95%CI: 7.7-76.7%) for patients with focal siderosis; and 74% (95%CI: 44.1-95.7) for patients with disseminated cSS (log-rank test: p=0.0031). In Cox regression models, any cSS and disseminated cSS were both independently associated with increased lobar ICH risk, after adjusting for ≥2 microbleeds and age (HR: 2.53; 95%CI: 1.05-6.15; p=0.040 and HR: 3.16; 95%CI: 1.35-7.43; p=0.008, respectively). These results remained consistent in sensitivity analyses including only patients with symptomatic lobar ICH at baseline.

Conclusions: Our findings indicate that cSS, particularly if disseminated, is associated with an increased risk of symptomatic lobar ICH in CAA. cSS may help stratify future bleeding risk in CAA, with implications for prognosis and treatment.

4.1 Introduction

Sporadic cerebral amyloid angiopathy (CAA) is a highly prevalent age-related small vessel disease[61] caused by amyloid–β deposition in cortical and leptomeningeal vessel walls.[28] CAA is a major cause of lobar intracerebral haemorrhage (ICH), particularly in elderly patients.[28, 33, 37, 242] Spontaneous ICH is one of the most catastrophic forms of stroke, with a high risk of recurrence;[220, 253, 254] CAA-related lobar ICH may carry a greater risk than deep ICH presumed due to hypertensive arteriopathy,[254, 255] but this is currently difficult to predict.

Predisposing factors for lobar ICH and lobar ICH recurrence in CAA include: APOE ε4 and ε2 alleles;[87] hemorrhagic neuroimaging markers of CAA such as lobar cerebral microbleeds (CMBs);[180] and anticoagulant or antiplatelet use.[227] Little is known about cortical superficial siderosis (cSS), a recently identified neuroimaging marker of CAA,[195] and the risk of subsequent ICH. cSS reflects linear blood residues in the superficial (subpial) layers of the cerebral cortex.[190] One likely mechanism leading to cSS is repeated episodes of haemorrhage into the subarachnoid space from brittle superficial cortical or leptomeningeal CAA-laden vessels, potentially heralding a high risk of future lobar ICH. A recent study showed that nearly 50% of CAA patients with cSS experienced intracranial haemorrhage over a period of 35 months,[246] but this study did not include patients without cSS as a control group.

We tested the hypothesis that in CAA patients, cSS, especially involving multiple sulci (reflecting more widespread or active disease), is associated with an increased risk of future symptomatic lobar ICH, in a European multicentre cohort study.

4.2 Patients and Methods

4.2.1 Study population and baseline data collection

We included consecutive patients diagnosed with CAA (according to the original Boston criteria,[202] – not including cSS as a criterion) at four stroke centres over defined time periods. The centres were: University College London Hospitals NHS Foundation Trust (London) (03/2003–09/2011), Addenbrooke's Hospital (Cambridge) (07/2002–03/2010), Cliniques Universitaires Saint Luc (Brussels) (12/2003–04/2010) and CHU Mont-Godinne UCL (08/2005–03/2009). At participating centres, MRI scanning is a routine investigation for cases of suspected CAA, unless there are contra-indications. Our inclusion criteria were: (1) patients fulfilling the original Boston criteria for CAA;[202] (2) available MRI sequences

including T2*-weighted gradient-recalled echo (T2*-GRE) and fluid attenuated inversion recovery (FLAIR) MRI; and (3) available follow-up information on symptomatic ICH, confirmed by neuroimaging. We included all patients with CAA, including survivors of spontaneous lobar ICH and those who, during investigation for other symptoms, were found to have strictly lobar CMBs (or asymptomatic lobar ICH). We excluded CAA patients without adequate MR imaging (n=26), or reliable follow-up data (n=37). Excluded subjects (n=63) did not differ significantly from those included in any baseline characteristics (all $p>0.05$).

Clinical data at the time of presentation (age, gender, vascular risk factors including hypertension, use of antithrombotics and previous symptomatic ICH) were obtained from prospective databases and by medical records review using standardised data collection forms. A clearly documented prior history of transient (≤24 hours), focal neurological episodes with no known alternative explanation other than CAA (e.g. structural brain lesion, atrial fibrillation, extracranial or intracranial stenosis) was ascertained by review of medical records.

4.2.2 Standard Protocol Approvals, Registrations, and Patient Consents

The study received ethical approval by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee, the Commission d'Ethique Biomedicale Hospitalo Facultaire of the Faculte de Medicine (Universite Catholique de Louvain), and the Comite d'ethique medicale of the CHU Mont Godinne UCL.

4.2.3 Magnetic resonance imaging acquisition and analysis

The MRI protocol was similar in each hospital. Imaging was at 1.5T field strength for all patients and included T1-weighted, T2-weighted, FLAIR and axial T2*-GRE (slice thickness 5 mm, repetition time 500–1,000 ms, echo time 15-70ms). Images were reviewed by a trained clinical research fellow blinded to clinical and follow-up data. The presence and distribution of CMBs were evaluated on T2*-GRE images using the Microbleed Anatomical Rating Scale.[238] Asymptomatic or symptomatic prior ICH (>5 mm in diameter on T2*-GRE MRI) was also noted.[198] cSS was defined as linear residues of chronic blood products in the superficial layers of the cerebral cortex showing a characteristic "gyriform" pattern of low signal on T2*-GRE images, without corresponding hyperintense signal on T1-weighted or

FLAIR images (i.e. without acute subarachnoid haemorrhage). We did not include cSS contiguous with any ICH. The distribution of cSS was classified as focal (restricted to ≤3 sulci) or disseminated (>3 sulci).[195] White matter changes were evaluated using the four-step simplified Fazekas rating scale (0-3: 0=no lesions; 1=focal lesions; 2=early confluent; 3=confluent).[239]

4.2.4 Follow-up

Follow-up data were obtained from a systematic review of multiple overlapping sources including prospective databases, medical records review (including discharge summaries, follow-up outpatient and general practitioner letters) and radiological databases, using standardized data collection forms. We collected information on clinically symptomatic ICH, defined as a symptomatic stroke syndrome associated with neuroimaging evidence of a corresponding ICH (>5 mm in diameter),[198] and death of any cause. Outcome events were assessed using all clinical, radiological, and pathological information available, blinded to the presence of cSS at baseline MRI. We determined whether the location of symptomatic ICH at follow-up corresponded to the anatomical distribution of cSS on baseline MRI.

4.2.5 Statistical analysis

We compared clinical and imaging characteristics of CAA patients with symptomatic lobar ICH during follow-up to patients without ICH, using chi-square tests and the Fisher's exact test for categorical variables, and two-sample t-tests or Mann-Whitney U-tests depending on the distribution of continuous variables. The reliability of rating for presence and category rating of cSS was assessed in a sample of MRI scans from probable CAA patients (n=48) by calculating Cohen's kappa and weighted kappa statistic respectively. We also compared basic clinical and imaging characteristics of patients with vs. without cSS. We determined the presence of cSS and disseminated cSS as univariate predictors of ICH risk using Kaplan-Meier plots with significance testing by the log-rank test. Survival time was calculated from date of baseline MRI scan until the date of symptomatic lobar ICH at follow-up or the last known date without the outcome event of interest. For individuals experiencing multiple lobar ICHs during follow-up, data were censored at time of first ICH. Data were also censored at the time of death from causes other than documented symptomatic ICH. Cox regression analysis was performed to calculate univariate hazard ratio (HR) as a measure of the effect size.

We estimated that our study would have a power of 88% to detect a difference in ICH risk between CAA patients with and without cSS, assuming 50% and 20% rates of ICH over four years respectively[180, 227, 246] (two-tailed test and with alpha values of 0.05).

According to the 'rule of 10' for developing proportional hazards models, we require approximately 10 outcome events for each potential covariate in multivariable analyses.[256, 257] Therefore, we investigated the effect the following prespecified potential predictors using Cox proportional-hazards model: presence of cSS or disseminated cSS, the presence of multiple CMBs (≥2), prespecified on the basis of the hypothesised effect on ICH risk from previous published series in CAA,[180, 227] and age, being an important potential confounder. As sensitivity analyses, we repeated all statistical tests in CAA patients with symptomatic lobar ICH at baseline, and those who had their MRI within six months of the index ICH. We also undertook exploratory multivariable models incorporating previous ICH as another potential confounding factor. The proportional hazard assumption was tested using graphical checks and Schoenfeld residuals-based tests.

A P-value≤0.05 was considered to be statistically significant. All analyses were performed using STATA (Version 11.2). The manuscript has been prepared with reference to the STROBE guidelines.[258]

4.3 Results

The final cohort consisted of 118 patients fulfilling the Boston diagnostic criteria[202] for CAA: 8 with pathologically-proven CAA, 86 with probable CAA and 24 with possible CAA. Of these, 104 (88%) patients presented with symptomatic lobar ICH at baseline, 8 with transient focal neurological episodes, 3 with cognitive decline, 1 with acute convexity subarachnoid haemorrhage and 2 with ischaemic stroke. The inter-rater agreement for the presence or absent of cSS was 89.6% (Cohen's kappa=0.79) and for cSS categories was 89.6% (weighted Cohen's kappa=0.75). Forty-one patients (43.8%, 95% CI: 26.2%-44.6%) had cSS at baseline. The presence of cSS was strongly associated with a prior history of transient focal neurological episodes (48.8% vs. 8.8%; p<0.001 respectively) and the presence of ≥5 CMBs (48.8% vs. 27.3%; p=0.019 respectively) (Appendix C Table 1). CAA patients with cSS were slightly older compared to patients without siderosis (mean age; 95%CI: 73.2; 70.4-76 *vs.* 70.3; 68.2-72.5 respectively, p=0.111).

During a median follow-up time of 24 months (interquartile range 9–44 months), 23 of 118 patients (19.5%, 95%CI: 12.8-27.8) experienced a symptomatic lobar ICH; four of these 23 patients experienced multiple (>1) sequential symptomatic lobar ICH during

follow-up. The characteristics of our cohort according to subsequent lobar ICH are shown in Table 4.1. Fourteen of 23 patients (61%) with lobar ICH during follow-up had cSS on baseline MRI: three had focal cSS and eleven had disseminated cSS (Table 4.1). For seven of these fourteen patients, the subsequent ICH was anatomically correlated with the area of cSS (Figure 4.1). Two of the four patients with multiple symptomatic lobar ICH at follow-up had disseminated cSS. One patient (without cSS at baseline) experienced a symptomatic deep (thalamic) ICH.

In the group of CAA patients without symptomatic lobar ICH at baseline (n=14; one patient with pathologically-proven CAA, 12 patients with probable CAA, and one with possible CAA), seven had cSS, which was disseminated in five cases. During a median follow-up time of 15.1 months (interquartile range 7.8–39.8), three of these patients experienced a symptomatic lobar ICH, all of whom had disseminated cSS at baseline scans.

Table 4.1 Characteristics and comparison of CAA patients according to symptomatic lobar intracerebral haemorrhage at follow-up. The p-values refer to differences between patients with vs. without symptomatic lobar intracerebral haemorrhage at follow-up. The median time between all baseline symptomatic ICH and first MRI was 17 days (IQR: 3-75.5 days).

Characteristics	No ICH at follow-up (n=95)	ICH at follow-up (n=23)	p-value
Age, mean (95% CI), years	70.4 (68.5-72.3)	75.2 (71.5-78.9)	0.026
Median follow-up time (months) (IQR range)	24.6 (9-51.3)	21.3 (6-38.5)	0.429
Sex, male (%)	51 (53.7)	10 (43.5)	0.379
Hypertension (%)	53 (63.5)	12 (52.2)	0.322
On antithrombotics at baseline (%)	36 (25.3)	6 (26.1)	0.936
Presented with symptomatic ICH at baseline (%)	84 (88.4)	20 (87)	1.000
Previous symptomatic ICH (%) (other than index event)	23 (24.1)	7 (30.4)	0.538
History of TFNE (%)	17 (17.9)	9 (39.1)	0.027
Superficial cortical siderosis (%)	27 (28.4)	14 (60.9)	0.003
Focal, ≤3 sulci (%)	11 (11.6)	3 (13)	1.000
Disseminated, >3 sulci (%)	16 (16.8)	11 (47.8)	0.002
Presence of CMBs (%)	62 (65.3)	18 (78.3)	0.231
Number of CMBs, median (IQR range)	2 (0-8)	3 (1-15)	0.412
≥2 CMBs (%)	54 (56.8)	15 (65.2)	0.465
≥5 CMBs (%)	32 (33.7)	9 (39.1)	0.623
Moderate-to-severe white matter changes (Fazekas score 2 or 3) (%)	41 (44.1)	11 (47.8)	0.747

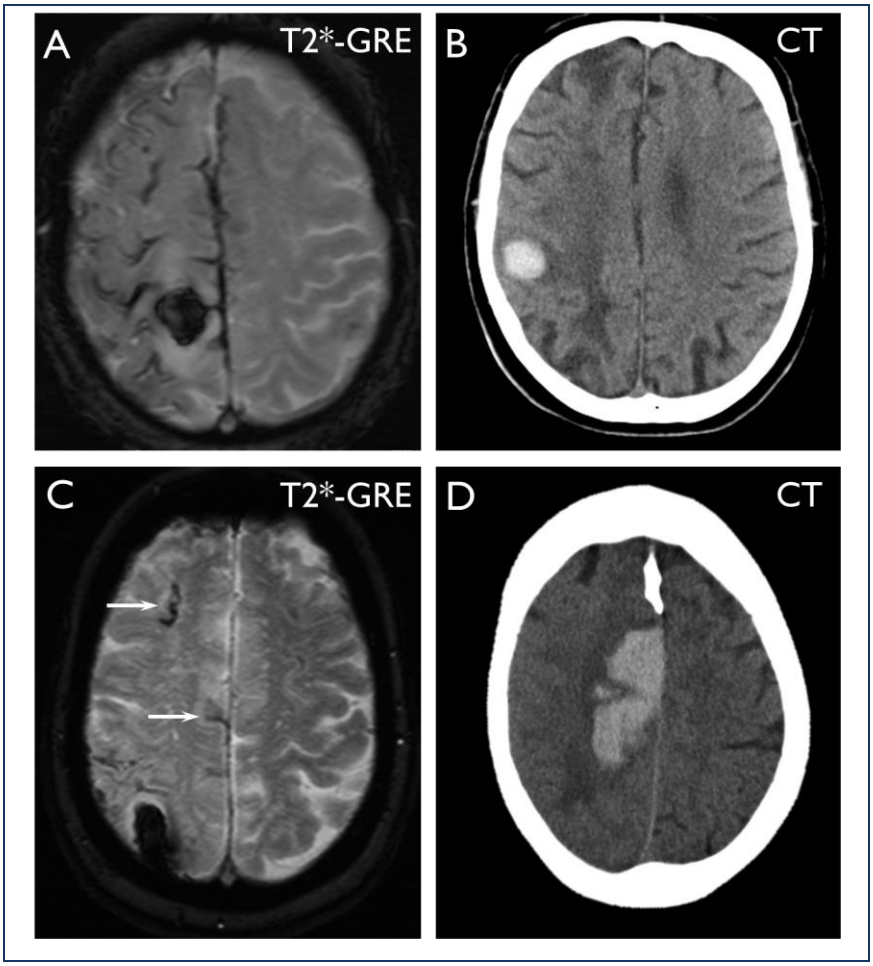

Figure 4.1 Subsequent intracerebral haemorrhage (ICH) at the site of pre-existing siderosis in two patients with cSS. 85-year-old female patient; time interval between baseline imaging (A) and incident ICH (B): 19 months. 80-year-old male patient, time interval between baseline imaging (C) and incident ICH (D): 35 months. A, C Baseline magnetic resonance imaging, T2*-weighted images; B, D Follow-up unenhanced computed tomography scans.

In Kaplan-Meier analysis the presence of cSS and disseminated cSS at baseline scans were predictors of time until ICH (p=0.0045 and p=0.0009 respectively, by the log-rank test) (Figure 4.2). The risk of symptomatic lobar ICH at 4 years of follow-up was 25% (95%CI: 7.6-28.3%) for patients without cSS at baseline, 28.9% (95%CI: 7.7-76.7%) for those with focal cSS, and 74% (95%CI: 44.1-95.7) for patients with disseminated cSS (p=0.0031 by the long-rank test for each category increase).

In univariate analysis, any cSS presence was a predictor of symptomatic lobar ICH (HR: 3.18; 95%CI: 1.37-7.39; p=0.007), with an increased risk associated with disseminated cSS (HR: 4.07 compared to no siderosis; 95%CI: 1.66-9.96; p=0.002). Focal cSS was associated with a non-significant increased hazard of subsequent ICH (HR: 1.91 compared to no siderosis; 95%CI: 0.51-7.19; p=0.340). For each increase in category of cSS (i.e. from no siderosis, to focal, and to disseminated siderosis) the associated HR was 2.06 (95%CI:

1.31-3.24; p=0.002). A history of previous symptomatic haemorrhage (before the index symptomatic ICH) (HR: 1.87; p=0.177), presence of CMBs (HR: 2.17; p=0.125), presence of ≥2 or ≥5 CMBs (HR: 1.55; p=0.318 and HR: 1.33; p=0.507 respectively) were not associated with future symptomatic lobar ICH in univariate analysis. Only age was associated with an increased hazard of subsequent ICH (HR: 1.07; p=0.006).

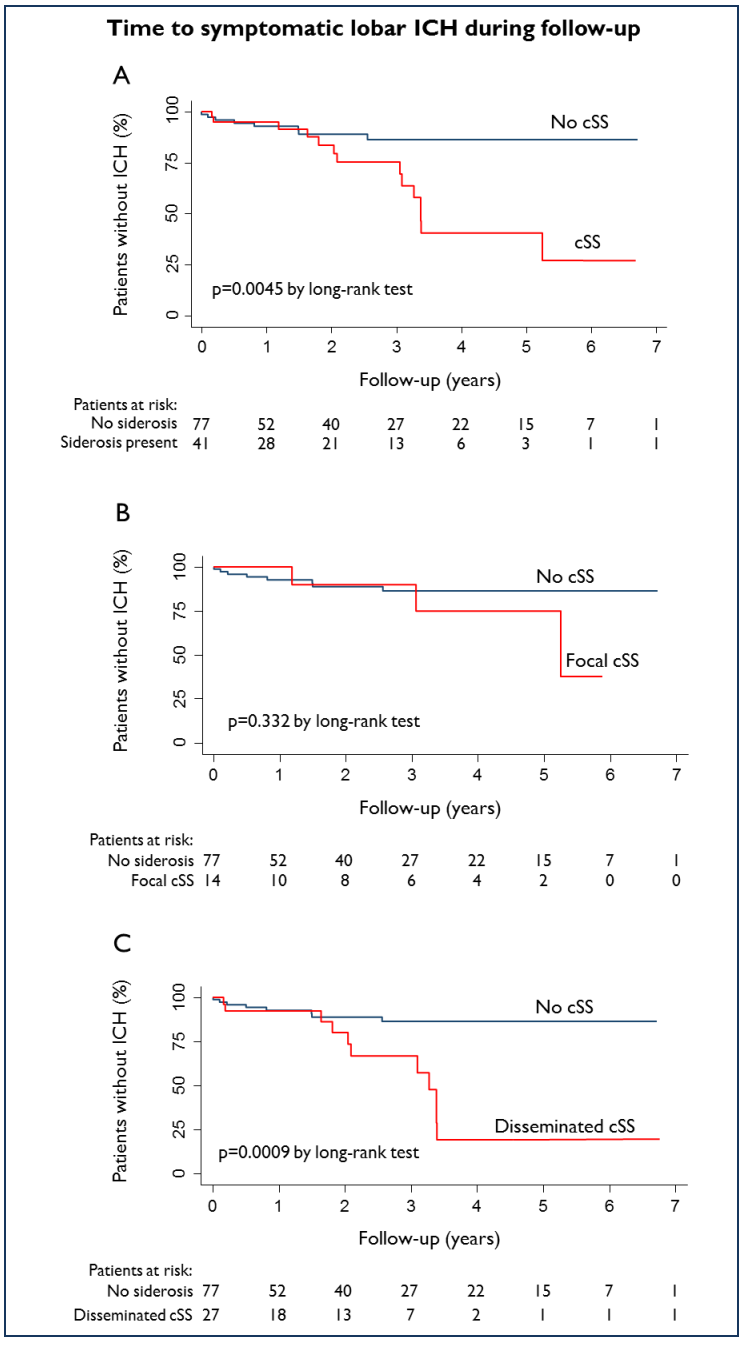

Figure 4.2 Kaplan-Meier estimates of progression to symptomatic lobar intracerebral haemorrhage (ICH) in the presence of: (A) cortical superficial siderosis (cSS); (B) focal cSS; and (C) disseminated (>3 sulci) cortical superficial siderosis in all patients with cerebral amyloid angiopathy (CAA). Testing of significance is by the log-rank test.

Table 4.2 Prespecified multivariate analyses of predictors of symptomatic lobar intracerebral haemorrhage during follow-up in patients with cerebral amyloid angiopathy. The models remain consistent if number of cerebral microbleeds (CMBs), presence of CMBs or ≥5 CMBs are included.

Model 1:	**HR (95%CI)**	**p-value**
Presence of cortical superficial siderosis	2.53 (1.05-6.15)	0.040
Presence of ≥2 CMBs	1.10 (0.45-2.71)	0.836
Age (for each year increase)	1.06 (1.01-1.11)	0.028
Model 2:	**HR (95%CI)**	**p-value**
Disseminated cortical superficial siderosis	3.16 (1.35-7.43)	0.008
Presence of ≥2 CMBs	1.07 (0.44-2.63)	0.876
Age (for each year increase)	1.07 (1.01-1.13)	0.017

Prespecified multivariable Cox regression models demonstrated that cSS and disseminated cSS were independently associated with increased risk of symptomatic lobar ICH at follow-up, after adjusting for the presence of ≥2 CMBs and age (Table 4.2). These effect sizes remained consistent in similar multivariable models controlling for CMBs number, CMBs presence and ≥5 CMBs. Our main results were also consistent in sensitivity analyses including cSS, CMBs, previous history of symptomatic ICH and age in multivariable models (Appendix C Table 2).

All the results of Kaplan-Meier and prespecified Cox regression analyses remained consistent in sensitivity analyses which included only CAA patients with symptomatic lobar ICH at baseline (Appendix C Figure 1) and patients who had their MRI within six months of the index ICH (data not shown).

4.4 Discussion

In this multicentre retrospective cohort study we found that cSS on T2*-GRE MRI (reflecting hemosiderin deposition in the subpial superficial cortical layers) significantly increases the risk of future symptomatic lobar ICH in patients with CAA.[202] The risk rate for ICH was greatest for patients with disseminated cSS, involving multiple sulci on baseline scans. These results remained consistent after adjusting for age, the presence of multiple (≥2) lobar CMBs (a haemorrhagic marker of CAA previously shown to influence the risk of ICH)[227], and previous symptomatic ICH prior to the index inclusion event. In a subanalysis

of those patients who presented with symptomatic ICH at baseline, cSS remained a predictor of ICH risk, consistent with the main analysis.

cSS is emerging as a common and characteristic feature of CAA.[28, 243] One study reported cSS in 60.5% (n=38; mean age 70±6.4 years) of patients with histopathologically proven CAA, compared with no control subjects with histopathologically proven non-CAA ICH (n=22; mean age 54±18 years).[195] Another recent study found cSS in 40% of patients with a clinic-radiological diagnosis of probable CAA-related ICH but only 5% of patients with purely deep ICH, presumed due to hypertensive arteriopathy.[259] Increasing data support the hypothesis that cSS might precede lobar ICH in patients with CAA.[191, 237, 260] In a recent retrospective study, 51 patients with cSS and no apparent cause other than CAA were identified through a single centre systematic database search and followed-up for a median of 35.3 months.[246] Over this period 24 patients (47.1%) experienced any new "intracranial haemorrhage" (ICH or acute convexity subarachnoid haemorrhage): 18 patients (35.3%) had an ICH, of which 13 were at the site of pre-existing siderosis.[246] This study was limited by the incomplete ascertainment of outcome intracranial bleeding events (without details of how many were symptomatic), and the lack of a comparison group without cSS at baseline.

Our larger study confirms that cSS is a risk factor for future symptomatic ICH in CAA, independent of multiple lobar CMBs. It is hypothesized that repeated episodes of haemorrhage from brittle superficial cortical or leptomeningeal CAA-affected vessels into the subarachnoid space leads to subpial haemosiderin deposition and cSS on MRI.[247] The finding of cSS without previous ICH, and its tendency to occur distantly from previous ICH, favour this "primary" mechanism,[195, 246] rather than leakage of blood into the subarachnoid space secondary to previous lobar "macro" ICH.[259] Consequently, cSS may be a marker of increased cortical and leptomeningeal small vessel fragility, high CAA disease activity, and vulnerability to subarachnoid bleeding, which in some circumstances may extend and develop into a lobar ICH.[248] Indeed, a neuropathological series of six autopsy cases of subcortical haematoma caused by CAA, showed that multiple leptomeningeal arteries can rupture into the subarachnoid space and the brain parenchyma.[248] This hypothesis is supported by the observation that symptomatic ICH has been noted at (or close to) the site of previous siderosis,[246] though we found that ICH only occurred at the site of cSS in 50% of cases. We found the highest ICH risk in patients with disseminated cSS, which may indicate widespread and numerous leptomeningeal vessels damaged by advanced CAA, providing multiple potential initiation sites for future ICH, increasing the probability of this outcome.[248] Further serial MRI studies will help to unravel the sequence of events and mechanisms linking CAA, cSS and lobar ICH, including asymptomatic bleeding.[252]

Assessment of the associations between ApoE genotype (which was not available in our cohort) and the extent or severity of CAA-related pathology in leptomeningeal vessels may also be of interest.

Although our results suggest an increased risk of subsequent ICH in CAA patients with cSS on MRI, antithrombotic drug use probably also plays a role in this risk by impairing haemostatic mechanisms.[28, 227, 255] In our retrospective cohort, it was not possible to systematically collect data on the use of antithrombotic drugs. However, routine clinical practice in all four centres in our study was to avoid of all antiplatelet agents (including aspirin) and anticoagulants in patients with suspected CAA, unless there was a very strong indication, so is unlikely to have contributed significantly to the outcome events in our study.

Our findings might have important implications for patients presenting with transient focal neurological episodes (sometimes called "amyloid spells"[249]) resembling transient ischaemic attacks, migraine auras or focal seizures,[167, 244, 249] which are increasingly recognized in CAA. It was recently reported that such attacks are often related to cSS and herald a high early risk of symptomatic lobar ICH (24.5% [95%CI: 15.8%–36.9%] at 8 weeks).[244] Thus, these types of attacks (which are often recurrent and stereotyped) should alert the clinician to the possibility of cSS; giving antithrombotic drugs to these patients due to misdiagnosis as TIA, could significantly increase the risk of serious future ICH.[252] Moreover, our current data suggest that disseminated siderosis may be a particular risk factor for future ICH in this situation.

Perhaps surprisingly, we did not find a significant association between multiple lobar CMBs and future ICH risk,[180, 227] which may reflect selection bias towards generally advanced disease with high prevalence of multiple lobar CMBs in our cohort. Although our study had adequate power to detect an increase in the risk of ICH in the presence of cSS, our sample size was not large enough to investigate additional potential baseline predictors of ICH (e.g. index ICH volume, ApoE genotype) or to investigate ICH risk in the subset of patients without lobar ICH at baseline. Other potential limitations include the retrospective design, and the potential of bias in our sample since the requirement for MRI may exclude more severe cases of ICH. Furthermore, a proportion of otherwise eligible patients did not have reliable follow-up data. Finally, we did not have pathological confirmation of CAA pathology; and acknowledge that the Boston criteria have imperfect specificity, especially for the "possible CAA" category.[202, 203]

Our findings nevertheless suggest that cSS is a useful independent prognostic marker of intracerebral bleeding risk in CAA. Larger cohorts are needed to confirm our results and

explore the potential implications for CAA treatment (e.g. avoiding antithrombotic agents in patients with disseminated cSS). cSS could also have implications for future disease-modifying treatments in CAA which may cause amyloid–β shifts between brain parenchyma and blood vessels (e.g. immunotherapy); CMBs have been considered a possible caution for such treatments,[261] but the role of cSS in this setting remains to be determined.

Chapter 5 MRI-visible perivascular spaces as a marker of underlying arteriopathy in intracerebral haemorrhage: Multicentre MRI cohort study

ABSTRACT

Objective: Small vessel disease (mainly hypertensive arteriopathy and cerebral amyloid angiopathy [CAA]) is an important cause of spontaneous intracerebral haemorrhage (ICH), a devastating and still poorly-understood stroke type. Enlarged perivascular spaces (PVS) are a promising neuroimaging marker of small vessel disease. Based on the underlying arteriopathy distributions, we hypothesised that severe centrum semiovale PVS are more common in lobar ICH attributed to CAA than other ICH. We evaluated PVS prevalence, severity and distribution, and their clinical-radiological associations.

Methods: Retrospective multicentre cohort study of 121 ICH patients. Clinical information was obtained using standardised forms. Basal ganglia and centrum semiovale PVS on T2-weighted MRI [graded 0-4 (>40 PVS)], white matter changes, cerebral microbleeds (CMBs) and lacunes were rated using validated scales.

Results: Patients with probable or possible CAA (n=76) had a higher prevalence of severe (>40) centrum semiovale PVS compared to other ICH patients (35.5% vs. 17.8%; p=0.041). In logistic regression age (OR: 1.43; 95%CI: 1.01-2.02; p=0.045), deep CMBs (OR: 3.27, 95%CI: 1.27-8.45; p=0.014) and mean white matter changes score (OR: 1.29; 95%CI: 1.17-1.43; p<0.0001) were independently associated with increased basal ganglia PVS severity; only age was associated with increased centrum semiovale PVS severity (OR: 1.50; 95%CI: 1.08-2.10; p=0.017).

Conclusions: PVS are common in ICH. Different mechanisms may account for PVS according to their anatomical distribution. Severe centrum semiovale PVS may be secondary to, and indicative of, CAA with value as a new neuroimaging marker. By contrast, basal ganglia PVS severity is associated with markers of hypertensive arteriopathy.

5.1 Introduction

Spontaneous (non-traumatic) intracerebral haemorrhage (ICH) is usually defined as ICH not due to any structural, vascular or other primary cause (e.g. tumour, arteriovenous malformation, aneurysm, cavernoma) and therefore attributed to intrinsic disease of the cerebral small vessels. Spontaneous ICH accounts for more than 75% of ICH in adults, leading to high rates of death and disability.[45] It results from the rupture of small arteries damaged by two main age-related cerebral small vessel diseases: hypertensive arteriopathy (including arteriolosclerosis) and cerebral amyloid angiopathy (CAA). Hypertensive arteriopathy typically affects the small perforating end-arteries of the deep grey nuclei and deep white matter and is an important cause of ICH in deep structures. CAA is characterised by the progressive deposition of amyloid-β in the walls of small vessels in the cerebral cortex, overlying leptomeninges and grey–white matter junction and is a major cause of lobar ICH in the elderly.[28] There is an urgent need to better understand the pathophysiology of cerebral small vessel disease and spontaneous ICH, to enhance diagnosis, prevention and treatment.

Perivascular spaces (also known as Virchow-Robin spaces) are interstitial fluid-filled [262] cavities surrounding the small penetrating vessels which function as the brain drainage system,[263] and have been recognized pathologically for many years. Enlarged perivascular spaces (PVS) can also be seen on MRI, and have attracted attention as a potential neuroimaging marker of small vessel disease.[30, 133] Although PVS are common finding in the elderly,[264, 265] and show consistent associations with other markers of small vessel damage [6], they have received relatively little attention to date–usually being considered unimportant or even normal. PVS are visible on T2-weighted cerebral MRI as characteristically small, high-signal areas in the basal ganglia and centrum semiovale.[30]

ICH is perhaps the most devastating clinical consequence of small vessel disease, yet PVS have not been systematically studied in this population. There is an urgent need to develop more sensitive diagnostic markers of the arteriopathies underlying ICH, particularly CAA.[28] In the present study our aims were to determine the prevalence, distribution and severity of PVS in ICH, and investigate their associations with other imaging markers of small vessel disease, and with presumed ICH aetiology. We hypothesized that, in patients with ICH: (1) PVS are common; (2) PVS severity is associated with other small vessel disease MRI markers including white matter changes, lacunes and cerebral microbleeds (CMBs); and (3) patients with strictly lobar ICH attributed to CAA have a higher

prevalence of severe PVS in the centrum semiovale compared to other ICH, due to the expected distributions of the underlying arteriopathies.

5.2 Patients and Methods

5.2.1 Study participants and data collection

We performed a retrospective analysis of patients with spontaneous ICH (defined as ICH without evidence for an underlying cause, except for small vessel disease) referred to four specialist stroke centres over defined time periods in the United Kingdom and Belgium. The hospitals were: University College London Hospitals NHS Foundation Trust (09/2004-06/2011), Cliniques Universitaires Saint Luc (Brussels) (12/2004–04/2008), CHU Mont-Godinne UCL (Belgium) (08/2005–06/2008) and Addenbrooke's Hospital (Cambridge) (07/2002–12/2009). At all participating centres, MRI scanning is a routine investigation for ICH, unless there are contra-indications. Cases were systematically ascertained using multiple overlapping methods from prospective clinical databases and radiological reports. A total of 377 patients with ICH were screened, of whom 129 were excluded because of diagnosis of non-spontaneous ICH (e.g. secondary to aneurysms, tumours, cavernomas etc.), and 127 because MRI with the necessary sequences of adequate quality was not available for analysis.

We categorised cases of spontaneous ICH as either strictly lobar, consistent with a diagnosis of CAA (clinically possible or probable CAA according to the Boston criteria[202]) or "other ICH" (i.e. deep ICH and mixed deep and lobar ICH). Demographic and clinical information (e.g. age at time of MRI, gender, vascular risk factors, use of antithrombotics etc.) was obtained from prospective databases and by medical records review using standardised data collection forms. Hypertension was defined as a history of hypertension, taking antihypertensive treatment or documented elevated blood pressure (systolic >150 or diastolic >95mmHg) prior to ICH, diabetes as ongoing use of a hypoglycaemic agent and smoking as history of tobacco use before admission.

The study received ethical approval by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee, the Commission d'Ethique Biomedicale Hospitalo Facultaire of the Faculte de Medicine (Universite Catholique de Louvain), and the Comite d'ethique medicale of the Cliniques Universitaires UCL de Mont Godinne.

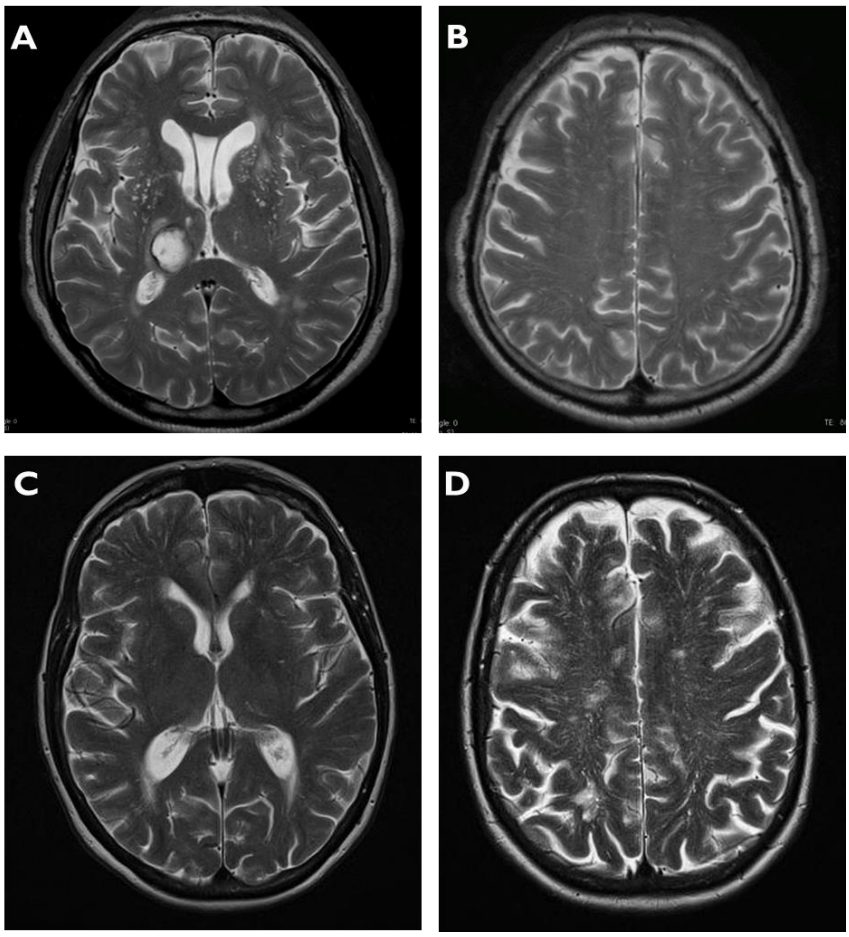

Figure 5.1 Axial T2-weighted MRI. (A, B) Dot-like hyperintensities characteristic of enlarged perivascular spaces (PVS) in the basal ganglia in a patient with spontaneous deep intracerebral haemorrhage (ICH). (C, D) Linear hyperintensities typical of PVS in the centrum semiovale in a patient with CAA-related lobar ICH. Note that deep brain regions (e.g. basal ganglia) are not affected by PVS (D). (For within group comparisons of the proportion of patients with centrum semiovale PVS vs. basal ganglia EPVS for lobar ICH and other ICH see Appendix D).

5.2.2 Magnetic resonance imaging acquisition and analysis

The MRI stroke protocol was similar in each hospital. Imaging was at 1.5T field strength and included axial T1-weighted, T2-weighted, coronal fluid-attenuated inversion recovery (FLAIR), axial diffusion-weighed imaging (DWI) and T2*-weighted gradient-recalled echo (T2*-GRE) sequences (Appendix D Table 1).

MR images were reviewed on medical imaging workstations, blinded to clinical details. The presence, number and distribution (lobar-including cerebellum, as suggested in the Boston criteria[202] and deep-including brainstem) of ICHs were recorded. The presence, number and distribution of CMBs were evaluated on T2*-GRE images using the Microbleed Anatomical Rating Scale.[238] White matter hyperintensities were rated on a four-point scale (0-3)[266] on coronal FLAIR, T1-weighted and T2-weighted MR images.

PVS were rated on axial T2-weighted MR images by a trained observer, using a validated visual rating scale.[30, 267] PVS were defined as small, sharply delineated structures of CSF intensity (or close to CSF intensity), measuring <3mm following the course of perforating or medullary vessels (Figure 5.1). Lacunes were distinguished form PVS by their larger size (>3mm), spheroid shape and surrounding hyperintensity on FLAIR. PVS were rated in basal ganglia and centrum semiovale regions. For both of these anatomical areas, the PVS rating categories applied were: 0=no PVS, 1=<10 PVS, 2=11-20 PVS, 3=21-40 PVS and 4=>40 PVS. The numbers refer to PVS on one side of the brain: after reviewing all relevant slices for the anatomical area being assessed, the slice and side with the highest number of PVS was recorded.[30, 267] In patients with other ICH, basal ganglia PVS were evaluated contralateral of the lesion if deep ICH was present. The assessment of PVS may have been influenced by the presence of confluent white matter changes; in such cases, as per guidance provided for the rating scale we used,[30] estimation was made of the closest PVS rating category, using the appearance of non-involved white matter, and cortical gray matter. Similarly, in cases of large lobar ICH, centrum semiovale PVS were assessed contralateral to the index ICH lesion; an estimation of the closest centrum semiovale PVS rating category ipsilateral to the lesion was made, and the highest number of PVS was recorded. Intra-rater reliability testing (n=30 scans) showed an excellent intra-rater Cohen's kappa value of 0.91 for basal ganglia PVS and 0.82 for centrum semiovale PVS.

5.2.3 Statistical analysis

We compared clinical and imaging characteristics of patients with strictly lobar ICH (attributed to CAA according to the Boston criteria) to those with other ICH, using chi-square tests and the Fisher's exact test for categorical variables, and two-sample t-tests or Mann-Whitney U-tests depending on the distribution of continuous variables. To test our hypothesis, we sought to compare the prevalence of severe centrum semiovale and basal ganglia PVS (>40 PVS) in the two ICH groups. However, since only one patient had severe basal ganglia PVS, in a post-hoc analysis we compared frequent-to-severe basal ganglia PVS (>20) in the two ICH groups. Both crude and age-adjusted severe PVS centrum semiovale prevalence rates are reported. For the logistic regression analysis, severity of PVS was trichotomized due to the small number of patients with no PVS or >40 PVS [1 (mild; 1 to 10), 2 (moderate; 11-20) and 3 (severe; over 21)]. Ordinal logistic regression analysis was used to identify predictors of increasing PVS severity in the two anatomical areas (centrum semiovale and basal ganglia) and for total brain PVS. Multivariable analysis was adjusted for age, lobar microbleeds, deep microbleeds, white matter changes score and lacunes, based

on the results of univariable analysis, plus other biologically plausible confounders, especially neuroimaging markers of small vessel disease. As a sensitivity analysis we repeated these analyses with the addition of other variables in our model (including sex, hypertension, CAA-group etc.). Using a likelihood ratio test, the assumption of proportional odds for the ordinal logistic regression models was met. A p-value ≤0.05 was considered to be statistically significant. All statistical analyses were carried out using STATA data analysis and statistical software (Version 11.2, StataCorp.).

5.3 Results

Patients with spontaneous ICH excluded from the study were not significantly different from those included in measures of ICH severity including: median (IQR range) ICH volume (10.5 cm^3; 2.93–18.81 cm^3 *versus* 12.84 cm^3; 4.05–25.31 cm^3; p=0.288), median (IQR range) Glasgow Coma Score on admission (14; 11–15 *versus* 15; 14–15; p=0.087) in patients excluded versus patients included respectively. Patients excluded had a higher prevalence of deep ICH (50.5% *versus* 27.4%, p=0.005). The final cohort consisted of 121 patients with ICH [mean age 69.4 (95%CI: 67.2–69.6); 69 (57%) male]; 76 (63%) had strictly lobar ICH, fulfilling the Boston criteria for probable (n=53) or possible (n=23). The clinical and radiological characteristics of the ICH cohort overall and according to ICH location are reported in Table 5.1. The median time from ICH to MRI was 10 days (interquartile range: 3-61 days).

5.3.1 Prevalence and distribution of PVS in ICH

Almost all patients had some degree of PVS (Table 5.1); the mean total score was 4.38 (95%CI: 4.18-4.58).

Patients with strictly lobar ICH had a significantly higher prevalence of severe PVS (>40 PVS) in the centrum semiovale compared to other ICH patients (35.5% vs. 17.8%, respectively; p=0.041) (Figure 5.2). The age-adjusted prevalences of severe PVS in the centrum semiovale are 29.3% (95% CI: 23.1% to 35.5%) in the strictly lobar ICH group vs. 13.5% (95% CI: 9.3% to 17.8%) in the "other ICH" group (p<0.05).

It was not possible to test for differences between the strictly lobar and other ICH groups in the prevalence of severe basal ganglia PVS since only one patient had severe basal ganglia PVS. However, in a post-hoc analysis the proportion of patients with frequent-to-

severe basal ganglia PVS (>20) was higher in those with other ICH than in strictly lobar ICH (20% vs. 4%, p=0.009).

There was no relationship between basal ganglia and centrum semiovale PVS severity (compared using chi square test across categories; Appendix D Table 2).

Table 5.1 Patient characteristics. P-values refer to differences between strictly lobar (clinically probable or possible CAA) and other ICH.

n (%)	Overall (N=121)	Strictly lobar ICH (N=76)	Other ICH (N=45)	P-value
Age, mean (95%CI), years	69.4 (67.2-69.6)	71.1 (68.4-73.8)	66.6 (62.8-72.4)	0.05
Sex, male	69 (57)	41 (54)	28 (62.2)	0.449
History of hypertension	86 (72.9)	49 (67.1)	37 (82.2)	0.090
Smoking history	39 (33.3)	22 (30.1)	17 (38.6)	0.419
Diabetes	20 (17.1)	11 (15.1)	9 (20.5)	0.459
On statins	26 (22)	15 (20.6)	11 (25)	0.648
On antithrombotics	38 (32.8)	27 (37)	11 (25.6)	0.226
First-ever ICH	68 (63)	40 (62.5)	28 (63.6)	1.000
Lacunar infarct	25 (49)	11 (37.9)	14 (63.6)	0.093
Multiple ICHs	47 (39.8)	34 (46.6)	13 (28.9)	0.081
Microbleeds presence	79 (67.5)	45 (62.5)	34 (75.6)	0.160
Lobar microbleeds	77 (64.7)	45 (62.5)	30 (68.2)	0.556
Deep microbleeds	28 (23.1)	0	28 (63.6)	<0.0001
Mean total white matter changes score (95%CI)	8.58 (7.59-9.57)	8.21 (6.95-9.46)	9.18 (7.53-10.83)	0.346
Mean total PVS score (95%CI)	4.38 (4.18-4.58)	4.29 (4.04-4.54)	4.53 (4.17-4.9)	0.255
Mean basal ganglia PVS score (95%CI)	1.50 (1.37-1.63)	1.32 (1.18-1.45)	1.8 (1.56-2.05)	0.0003
Mean centrum semiovale PVS score (95%CI)	2.85 (2.72-3.05)	2.97 (2.76-3.19)	2.73 (2.47-2.99)	0.1590
PVS centrum semiovale				
Mild: 1-10	7 (5.8)	4 (5.3)	3 (6.7)	0.338†
Moderate: 11-20	36 (29.8)	21 (27.6)	15 (33.3)	
Frequent: 21-40	43 (35.5)	24 (31.6)	18 (40)	
Severe: >40	35 (28.9)	27 (35.5)*	8 (17.8)*	
PVS basal ganglia				
No EPVS	2 (1.7)	2 (2.6)	0 (0)	0.005†
Mild: 1-10	70 (57.9)	51 (67.1)	19 (42.2)	
Moderate: 11-20	37 (30.6)	20 (26.3)	17 (37.8)	
Frequent: 21-40	11 (9.1)	3 (4)	8 (17.8)	
Severe: >40	1 (0.8)	0 (0)	1 (2.2)	

†p-value for trend across groups

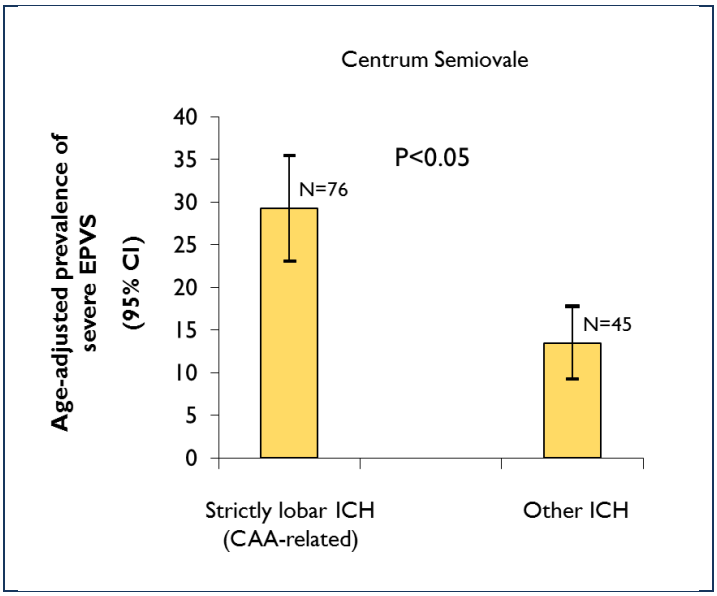

Figure 5.2 Age-adjusted prevalence of severe centrum semiovale PVS (>40 EPVS) in patients with strictly lobar intracerebral haemorrhage ICH, attributed to cerebral amyloid angiopathy (CAA), compared to other ICH. Age was used as a continuous variable.

Table 5.2 Univariable (unadjusted) ordinal logistic regression analysis showing predictors of increased basal ganglia and centrum semiovale PVS severity in the whole cohort.

(Yes vs. No)	Basal ganglia PVS		Centrum semiovale PVS	
	OR (95%CI)	**P-Value**	**OR (95%CI)**	**P-Value**
Age (per 10 years older)	**1.41 (1.02-1.95)**	**0.036**	1.36 (0.98-1.87)	0.065
Sex, male	0.53 (0.26-1.10)	0.088	0.56 (0.27-1.18)	0.128
History of hypertension	1.05 (0.57-2.33)	0.469	0.68 (0.28-1.63)	0.381
Smoking history	**2.89 (1.35-6.18)**	**0.006**	1.20 (0.53-2.71)	0.666
Diabetes	1.26 (050-3.16)	0.622	1.11 (0.39-3.13)	0.851
On statins	0.71 (0.29-1.72)	0.441	0.74 (0.31-1.81)	0.514
On antithrombotics	0.62 (0.28-1.38)	0.240	0.70 (0.31-1.57)	0.387
First ever ICH	0.64 (0.30-1.37)	0.248	1.07 (0.47-2.46)	0.866
Other ICH (non-CAA aetiology)	**3.58 (1.70-7.54)**	**0.001**	0.74 (0.35-1.57)	0.431
Lacunar infarct	2.75 (0.93-8.14)	0.068	2.84 (0.87-9.29)	0.083
Multiple ICHs	0.71 (0.34-1.47)	0.350	0.87 (0.41-1.86)	0.724
Microbleeds presence	**2.29 (1.01-5.21)**	**0.048**	1.62 (0.73-3.56)	0.233
Strictly lobar microbleeds	0.99 (0.48-2.04)	0.985	1.59 (0.73-3.47)	0.246
Lobar microbleeds	1.81 (0.82-3.99)	0.140	1.89 (0.87-4.13)	0.109
Deep microbleeds	**2.25 (1.01-4.98)**	**0.046**	0.97 (0.41-2.26)	0.937
Mean total WMH score (for each unit increase)	**1.23 (1.13-1.33)**	**<0.0001**	1.01 (0.94-1.08)	0.772

Table 5.3 Multivariable (adjusted) ordinal logistic regression analysis showing predictors of increased basal ganglia and centrum semiovale EPVS severity in the whole cohort.

	Basal ganglia PVS		Centrum semiovale PVS	
	OR (95%CI)	**P-Value**	**OR (95%CI)**	**P-Value**
Age (per 10 years older)	**1.43 (1.01-2.02)**	**0.045**	**1.50 (1.08-2.10)**	**0.017**
Lobar microbleeds	0.43 (0.16-1.20)	0.107	1.92 (0.74-4.96)	0.180
Deep microbleeds	**3.27 (1.27-8.45)**	**0.014**	0.91 (0.35-2.38)	0.850
Mean total WMH score (for each unit increase)	**1.29 (1.17-1.43)**	**<0.0001**	0.99 (0.91-1.08)	0.856
Lacunar infarct	1.00 (1.00-1.01)	0.362	1.00 (0.99-1.00)	0.918

5.3.2 Predictors of increased PVS severity in logistic regression

In univariable ordinal logistic regression analysis, significant predictors of increasing PVS severity in the basal ganglia were age, smoking history, the presence of CMBs, presence of deep CMBs, presence of lacunes, mean white matter changes score and other ICH (non-CAA aetiology) (Table 5.2). In multivariable logistic regression analysis (Table 5.3) age (OR: 1.43, per 10 years increase; 95%CI: 1.01-2.02; p=0.045), presence of deep CMBs (OR: 3.27, 95%CI: 1.27-8.45; p=0.014) and mean white matter changes score (OR: 1.29, per unit increase; 95%CI: 1.17-1.43; p<0.0001) were independent predictors of increased basal ganglia PVS severity, after adjusting for the presence of lobar CMBs and lacunes.

No clinical or radiological variables were found to be significantly associated with increasing PVS severity in the centrum semiovale, at the 5% level, in univariable analysis; age was marginally significant. In multivariable analysis, age was the only significant independent predictor of increasing PVS severity in the centrum semiovale: for every 10 years increase in age the odds of having an increase in centrum semiovale PVS was 1.50 (95%CI: 1.08-2.10; p=0.017).

5.4 Discussion

In this multicentre retrospective study we found that the distribution of severe PVS differed according to the location of ICH: severe centrum semiovale PVS were twice as common in patients with strictly lobar (i.e. probable or possible CAA) than in those with other ICH. By contrast, the ICH patients not fulfilling these criteria for CAA more often had frequent-to-severe basal ganglia PVS compared to strictly lobar ICH patients. In the whole cohort, we

found that age, white matter changes and deep CMBs were independently associated with basal ganglia PVS severity, but only age was independently associated with increased centrum semiovale PVS severity. Our study thus provides new evidence that the distribution of severe PVS reflects the underlying arteriopathy type in spontaneous ICH.

We found PVS in almost every participant in our study, and moderate to severe centrum semiovale and basal ganglia PVS in more than 90% and 40% of them respectively. To our knowledge, no previous imaging study has investigated the prevalence and distribution of PVS in spontaneous ICH. A recent prospective study which evaluated PVS in patients with acute lacunar stroke (n=129), cortical stroke (n=124) and age-matched non-stroke controls (n=97), found that PVS were more prevalent in small vessel (lacunar) ischaemic stroke than large vessel stroke.[30] In this study, Doubal and colleagues[30] used the same PVS rating scale, applied to T2-weighted images, as in our study, thus allowing comparisons to be made. The mean total PVS score in our ICH cohort (4.38, 95% CI: 4.18-4.58) is close to that of patients with lacunar stroke (3.81, SD: 1.76) but higher than non-stroke controls (1.02, SD: 0.89).[30] However, the absence of a non-ICH control group in our study makes it difficult to directly compare PVS counts with other patient groups.

Although PVS have not been studied in ICH cohorts, previous pathological and neuroimaging studies in other patient populations have shown a significant association between hypertension, white matter changes, and PVS.[24, 268] The associations we found between the severity of basal ganglia and total PVS with the severity of white matter changes further add to evidence that PVS are an imaging manifestation of cerebral small vessel disease rather than an incidental finding. The association of PVS with white matter changes seems consistent across different populations including vascular dementia,[269] Alzheimer's disease and mild cognitive impairment. A large population-based study also reported an association between the degree of PVS and the volume of white matter changes, as well as with the prevalence of lacunes.[270]

We also found that PVS associations vary according to their distribution, suggesting different pathophysiological mechanisms relevant to the underlying arteriopathy in ICH. Basal ganglia PVS were independently associated with age, white matter changes burden and deep CMBs, suggesting hypertensive arteriopathy as a possible underlying mechanism. It is likely that at least some white matter changes share a common pathophysiological mechanism with PVS, perhaps related to blood-brain barrier disruption, a potentially key pathogenic process in cerebral small vessel disease.[133] Indeed, it has been suggested that white matter changes tend to form around PVS[133] and that PVS may be an early imaging marker of small vessel disease and blood-brain barrier alteration. By contrast, in the

present study, PVS in the centrum semiovale were only associated with age, and not with other imaging or clinical factors. In the study by Doubal et al[30] in patients with minor ischaemic stroke, total PVS were also associated with white matter changes after adjusting for age, the presence of stroke and vascular risk factors ($p<0.001$); centrum semiovale PVS were not associated with any explanatory variables, in keeping with our findings.[30] It is thus possible that a distinct pathophysiological process other than hypertensive arteriopathy contributes to the development of centrum semiovale PVS: CAA is a likely candidate, a hypothesis consistent with evidence that advancing age is the strongest clinical risk factor for sporadic CAA.[28] Although we did not find a significant association between PVS severity in the centrum semiovale and the presence of lobar microbleeds (a putative neuroimaging marker of CAA) at the 5% level, the odds ratio suggests a possible effect (OR: 1.92; 95%CI: 0.74-4.96; Table 3), our ability to detect any association may be limited by our sample size and the high prevalence of hypertension, which can also contribute to lobar CMBs. In addition, the absence of association between basal ganglia and centrum semiovale PVS severity further supports the notion that PVS in these two anatomical compartments are related to different pathophysiological processes.

Severe PVS in our ICH cohort showed a different anatomical pattern depending on the location and the presumed small vessel arteriopathy underlying ICH. In patients with strictly lobar, presumed CAA-related ICH the centrum semiovale was more often affected by severe PVS compared to other ICH patients. A predilection of severe PVS for the centrum semiovale most parallels the topography of CAA pathology, since amyloid-β is deposited primarily in cortical and leptomeningeal small vessels, largely sparing small vessels in the basal ganglia.[28] Moreover, several lines of evidence suggest that perivascular spaces have a key role in the pathophysiology of CAA.[28] It is hypothesized that an important mechanism underlying vascular amyloid-β deposition is impaired clearance of amyloid-β along perivascular drainage pathways.[28] As amyloid-β is deposited in the walls of small arteries it could block perivascular drainage, leading to dilation of perivascular spaces downstream in the underlying deep white matter, even in regions not directly affected by CAA pathology. This regional preference for severe PVS further supports our hypothesis that severe centrum semiovale PVS are at least partly related to CAA, whereas basal ganglia PVS are most likely related to hypertensive arteriopathy affecting deep perforators. Severe centrum semiovale PVS may therefore be a potential useful new neuroimaging marker of CAA, in an appropriate clinical context. The diagnosis of CAA currently depends on the radiological demonstration of multiple strictly lobar haemorrhages; existing criteria have high specificity but limited sensitivity.[202, 203] Whether severe PVS presence and topography might usefully

improve the sensitivity for current diagnostic criteria for CAA[202] requires further study with pathological verification of CAA.

Our multicentre study has several strengths including the systematic evaluation of MRI scans by trained raters using validated scales for a range of imaging markers of small vessel disease, and the use of a validated PVS rating scale.[30] A potential limitation is the lack of pathological confirmation of the CAA pathology as a cause of ICH, in view of the imperfect sensitivity and specificity of the Boston criteria (particularly their overall sensitivity and the specificity of the "possible CAA" category).[202] We may thus have misclassified some patients as CAA-related ICH when their ICH was related to hypertensive arteriopathy. It is also likely that some of the patients with "other ICH" (e.g. spontaneous deep ICH), many of whom had lobar CMBs, not classified as CAA-related might in fact have some degree of CAA pathology as well as hypertensive arteriopathy. However, any misclassification of patients would tend to bias toward a null result (no between-group differences in PVS severity or distribution), suggesting that PVS may in fact be a more powerful marker for CAA than we have been able to demonstrate here. It was not possible to blind the PVS rater to ICH location, potentially introducing a bias towards the study hypothesis. We also acknowledge the potential of bias in our sample as MRIs were done as part of routine clinical care and many patients were excluded from this analysis because of inadequate MRI data. Finally, due to the retrospective and cross-sectional nature of our study we were not able to determine the prognostic significance of PVS in this cohort.

In summary, we found that PVS are present in almost all patients with spontaneous ICH, but with evidence for different underlying mechanisms according to their distribution. Basal ganglia PVS severity is associated with markers of hypertensive arteriopathy including white matter changes and deep CMBs, whilst centrum semiovale PVS are associated with age and are more often severe in patients with strictly lobar ICH attributed to CAA. A predilection of severe PVS for the centrum semiovale rather than the basal ganglia may therefore be secondary to, and indicative of, CAA. Further studies with pathological verification are needed to determine the relevance of PVS for improving the sensitivity of CAA diagnostic criteria. Longitudinal studies are required to explore whether PVS are an early feature of the small vessel disease underlying ICH, their change over time, and prognostic implications for clinical outcome.[267, 271]

Chapter 6 MRI-visible perivascular spaces as a neuroimaging marker of cerebral amyloid angiopathy: MRI-histopathological study

ABSTRACT

Objective: We investigated whether severe MRI-visible Perivascular Spaces (PVS) in the cerebral hemisphere white matter (centrum semiovale) are more common in patients with pathology-proven CAA vs. those with pathology-proven non-CAA related intracerebral haemorrhage (ICH).

Methods: Using a validated 4-point scale on axial T2-weighted MRI, we compared PVS in patients with pathology-proven CAA to those with spontaneous ICH but no histopathological evidence of CAA. In a preliminary analysis restricted to patients with T2*-weighted gradient-recalled echo MRI, we also investigated whether including severe centrum semiovale PVS increases the sensitivity of existing diagnostic criteria for probable CAA.

Results: Fourteen CAA patients and 10 non-CAA related ICH patients were included. Eight of the CAA patients were admitted for symptomatic spontaneous lobar ICH, one because of ischaemic stroke, one with transient focal neurological episodes, and four due to cognitive decline. Severe (>20) centrum semiovale PVS were more frequent in patients with CAA compared to controls (12/14 [85.7%; 95%CI: 57.2-98.2%] vs. 0/10 [one-sided 95%CI: 0-30.8%], p<0.0005); this was robust to adjustment for age. The original Boston criteria for probable CAA showed a sensitivity of 76.9% (95% CI: 46.2-95%), which increased to 92.3% (95% CI: 64-99.8%), without loss of specificity, after including severe centrum semiovale PVS.

Conclusions: Severe centrum semiovale PVS on MRI may be a promising new neuroimaging marker for the *in vivo* diagnosis of CAA. However, our findings are preliminary and require confirmation and external validation in larger cohorts of pathology-proven CAA.

6.1 Introduction

Sporadic cerebral amyloid angiopathy (CAA),[2] caused by progressive vascular amyloid-β deposition in small cortical and leptomeningeal vessels, is a major and increasingly frequent cause of lobar intracerebral haemorrhage (ICH) and cognitive impairment in older patients.[28, 37, 242] CAA is associated with neuroimaging markers including lobar ICH and strictly lobar cerebral microbleeds, which allow its clinical-radiological diagnosis using the Boston criteria.[28, 202, 203] However, these criteria have limited sensitivity in some studies; new non-invasive diagnostic markers of CAA are therefore needed, particularly to identify early disease.[28, 242]

Recently, perivascular spaces (PVS) have emerged as a potential MRI marker of the presence and severity of cerebral small vessel disease.[30, 31, 272-275] Perivascular spaces are interstitial fluid-filled cavities surrounding small penetrating vessels, which function to allow interstitial fluid and solute efflux from the brain.[129, 263, 276] CAA almost invariably accompanies Alzheimer's disease (AD), and in this setting vascular amyloid-β is associated with enlargement of perivascular spaces.[38, 128, 132, 277] However, whether PVS are associated with sporadic CAA is not known. We hypothesized that progressive amyloid-β deposition in CAA impairs interstitial fluid drainage, causing MRI-visible severe PVS in the cerebral hemisphere white matter (centrum semiovale). To test this hypothesis, we compared the prevalence of severe centrum semiovale PVS in patients with pathology-proven sporadic CAA compared to patients with pathology-proven non-CAA related spontaneous ICH. In a preliminary analysis, we also investigated whether including severe centrum semiovale PVS increases the sensitivity of existing diagnostic criteria for CAA.[202]

6.2 Methods

6.2.1 Study participants and data collection

We included all eligible patients from four stroke centres identified retrospectively by a systematic keyword search of pathology reports (Appendix E Figure 1). Cases were defined as subjects with both pathology-proven CAA (from routinely collected brain biopsy, biopsy at haematoma evacuation or autopsy) and adequate T2-weighted MRI sequences. Controls were defined as subjects with spontaneous symptomatic ICH, no pathological evidence of CAA, and adequate T2-weighted MRI.

6.2.2 Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee and the Commission d'Ethique Biomedicale Hospitalo Facultaire of the Faculte de Medicine (Universite Catholique de Louvain).

6.2.3 Pathological analysis

Brain biopsies or haematoma evacuation samples were fixed in 10% formalin, embedded in paraffin and processed for paraffin sectioning. Routine haematoxylin-eosin staining was performed for morphological assessment and the presence or absence of vascular amyloid-β deposition was confirmed by immunohistochemical detection. Samples not containing any assessable vessels were excluded from analysis. We assessed CAA presence and severity in all available vessels (in all cases from solid tissue fragments except in 1 case from isolated vessels); we also determined, wherever possible, whether vessels were leptomeningeal or parenchymal. CAA severity was graded using the modified Vonsattel grading system;[67, 72] all included CAA cases had a severity of at least grade 2 (i.e. replacement of the whole vessel wall by amyloid-β) in line with previous recommendations.[67] Non-CAA was defined as absence of vascular amyloid-β deposition.

6.2.4 Magnetic resonance imaging data and analysis

Mandatory imaging included axial T2-weighted sequences in all patients (slice thickness: 5mm, field strength: 1.5T) and, whenever available, T2*-weighted gradient-recalled echo (T2*-GRE) sequences. MR images were reviewed blinded to clinical details, histopathological diagnosis and microbleeds status. PVS were defined and rated on T2-weighted MR images, according to STandards for ReportIng Vascular changes on nEuroimaging (STRIVE),[31] by a trained observer 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 and centrum semiovale (cerebral hemisphere white matter).[30, 267] Intra-rater reliability testing of the PVS scale using an independent dataset of ICH scans (n=30) showed an intra-rater Cohen's kappa of 0.91 for basal ganglia PVS and 0.82 for centrum semiovale PVS.

6.2.5 Statistics

We compared clinical characteristics between the patients with vs. without CAA on neuropathology, using the Fisher's exact test for categorical variables, and Mann-Whitney U-test for age (not normally distributed continuous variable). We pre-specified a definition of severe centrum semiovale PVS as >20 (Grade 3). Although arbitrary, this definition is in line with the most severe category of white matter PVS used in a previous study (and found to relate to vascular risk factors),[270] and provided two numerically-balanced categories consistent with our aim of increasing the sensitivity of CAA diagnosis. We compared both the crude and age-standardized prevalence of severe PVS between groups using Fisher's exact test. We calculated the sensitivity and specificity after the addition of severe centrum semiovale PVS to the original Boston diagnostic criteria for CAA (including strictly lobar cerebral microbleeds)[202] in line with Standards for Reporting of Diagnostic Accuracy (STARD) guidelines.[278] The histopathological information was not taken into account for the classification. The analysis for clinically probable CAA was restricted to patients with T2*-GRE MRI sequences available. Significance level was set at 0.05 for all analyses. Statistical analyses were carried out using STATA (Version 11.2, StataCorp.).

6.3 Results

Fourteen CAA patients and 10 non-CAA ICH patients were included (Table 6.1) out of 56 screened; 30 patients were excluded because of no MRI available, 1 non-CAA patient because of no evidence of ICH on MRI, and another non-CAA patient in whom the tissue sample contained no assessable vessels. Eight of the CAA patients were admitted with symptomatic spontaneous lobar ICH, one with ischaemic stroke, one with transient focal neurological episodes, and four with cognitive decline (one of these also had radiological evidence of a previous ICH). All non-CAA patients were admitted with symptomatic spontaneous ICH. Twenty-three of the 24 available pathological samples contained at least 2 assessable parenchymal or leptomeningeal vessels. There were 23 solid tissue samples including vessels, and one sample including only isolated vessels. All 14 CAA cases contained >10 assessable vessels; 12 cases with both cortical and leptomeningeal, and 2 with only leptomeningeal vessels. Of the 10 non-CAA cases, 4 contained >10 assessable vessels, while the other 6 cases contained a median number of 2 assessable vessels (Interquartile Range: 2-3); 1 case contained both cortical and leptomeningeal, 5 only cortical, and 3 only leptomeningeal vessels; in the case containing only isolated vessels, their

Table 6.1 Patient and imaging characteristics.

n (%)	CAA† (N=14)	Non-CAA ICH† (N=10)	p-value
Age, median (IQR), years	66.9 (65.3-71.4)	56.9 (48.8-66.5)	0.035*
Sex, female	11 (78.6%)	5 (50%)	0.204
History of hypertension	4 (28.6%)	3 (30%)	1.000
Presence of radiological ICH	9 (64.3%)	10 (100%)	0.053
Lobar ICH on MRI (symptomatic or asymptomatic)	9 (64.3%)	8 (80%)	0.653
Deep ICH on MRI	0 (0%)	2 (20%)	0.163
Cerebral microbleeds (T2* GRE MRI was available in 13 CAA and 8 non-CAA patients)			
Cerebral microbleeds presence	11/13 (84.6%)	5/8 (62.5%)	0.325
Lobar (cortical-subcortical) CMBs	11/13 (84.6%)	1/8 (12.5%)	0.002*
Deep CMBs	1/13 (7.7%)	2/8 (25%)	0.531
Cerebellar CMBs	0/13 (0%)	1/8 (12.5%)	0.381
PVS centrum semiovale			
Severe PVS (>20)	12(85.7%)	0 (0%)	<0.0005*
No PVS	0 (0%)	1 (10%)	0.417
1-10 PVS	0 (0%)	4 (40%)	0.020*
11-20 PVS	2 (14.3%)	5 (50%)	0.085
21-40 PVS	6 (42.9%)	0 (0%)	0.024*
>40 PVS	6(42.9%)	0 (0%)	0.024*
PVS basal ganglia			
1-10 PVS	13 (92.9%)	9 (90%)	1.000
11-20 PVS	1 (7.1%)	1 (10%)	1.000
21-40 PVS	0	0	-
>40 PVS	0	0	-

†CAA cases: 8 haematoma evacuations, 5 brain biopsies and 1 autopsy; non-CAA ICH: 10 haematoma evacuations; CAA severity grading: Vonsattel Grade 2 (n=6), Grade 3 (n=6) and Grade 4 (n=2).[67]

origin could not be determined. The amount of available pathological tissue was generally greater in the CAA cases than the non-CAA cases.

Severe (>20) centrum semiovale PVS were detected in 85.7% (95%CI: 57.2-98.2%) of patients with histopathologically-confirmed CAA, but in none (one-sided 95%CI: 0-30.8%) of the controls with histopathologically confirmed non-CAA ICH (p<0.0005) (Figure 6.1). The age-standardised prevalences of severe centrum semiovale PVS were 85.7% (95%CI: 65.7%-100%) in the CAA group *versus* 0% in the non-CAA ICH group. The prevalence of the most severe category of centrum semiovale PVS (>40) was also higher in CAA than

non-CAA ICH (Table 6.1). Within the CAA group, 7/8 (87.5%; 95%CI: 47.4%-99.7%) patients who presented with symptomatic lobar ICH had severe centrum semiovale PVS ($p<0.0005$ compared with the non-CAA ICH group). There was no difference in the prevalence of any basal ganglia PVS category between the CAA and non-CAA ICH group.

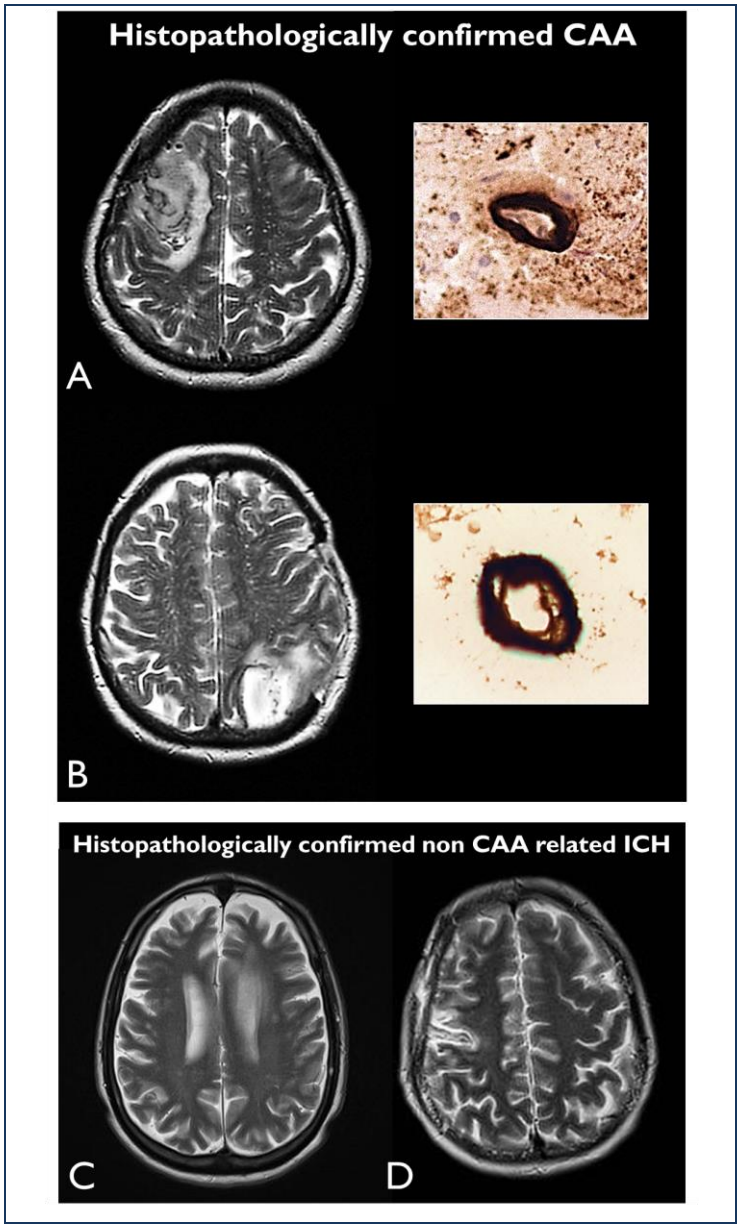

Figure 6.1 MRI-visible white matter perivascular spaces in CAA and non-CAA ICH. Perivascular spaces (PVS) are defined as small, sharply delineated structures of (or close to) cerebrospinal fluid intensity, measuring <3mm following the course of perforating or medullary vessels, in basal ganglia and centrum semiovale. In (A) and (B) the left panels show dot-like hyperintensities (florid PVS) on representative axial T2-weighted MRI at 1.5 Tesla in the centrum semiovale, in patients with histopathologically confirmed cerebral amyloid angiopathy (CAA); the right panels show the corresponding pathology (immunostaining for A β) with dense amyloid deposition spanning the entire vessel wall of a cortical (A, right panel) and a leptomeningeal arteriole (B, right panel). (C) and (D) show axial T2-weighted MRI from two of the non-CAA cases, showing a paucity of centrum semiovale PVS.

Table 6.2 Diagnosis of cerebral angiopathy (CAA) using the original Boston criteria (including strictly lobar microbleeds) with and without the inclusion of severe centrum semiovale PVS (>20 PVS). Only cases with T2*-GRE were included in this analysis (n=21). Inclusion of severe centrum semiovale PVS resulted in the diagnostic upgrading of three patients: two from possible to probable CAA and one from non-CAA to possible CAA.

Clinical Diagnosis	Original Boston criteria			With inclusion of severe centrum semiovale PVS		
	n	Pathologic diagnosis		n	Pathologic diagnosis	
		CAA (+)	CAA (-)		CAA (+)	CAA (-)
Probable CAA	11	10	1	13	12	1
Non-probable CAA	10	3	7	8	1	7
-Possible CAA	4	2	2	3	1	2
-Non CAA	6	1	5	5	0	5
Total	21	13	8	21	13	8

T2*-GRE MRI was available in 13 CAA and 8 non-CAA ICH patients. Adding severe centrum semiovale PVS (>20 PVS) to the original Boston criteria for CAA resulted in the diagnostic upgrading of three patients (Table 6.2). The original Boston criteria showed a sensitivity of 92.3% (95% CI: 64-99.8%) for possible or probable CAA diagnosis, while inclusion of severe centrum semiovale PVS increased their sensitivity to 100% (95% CI: 75.3-100%), without loss of specificity (62.5%; 95% CI: 24.5-91.5%). For probable CAA the original Boston criteria had a sensitivity of 76.9% (95% CI: 46.2-95%); inclusion of severe centrum semiovale PVS increased this to 92.3% (95% CI: 64-99.8%). The specificity for probable CAA was 87.5% (95% CI 47.3–99.7%) for both criteria.

6.4 Discussion

We have shown that severe PVS in the centrum semiovale white matter are more common in subjects with pathology-proven sporadic CAA than control non-CAA subjects. These findings suggest that severe centrum semiovale PVS, in this patient population, may be a promising new neuroimaging marker to improve the sensitivity of *in vivo* diagnosis of CAA. However, the sensitivity and specificity of severe centrum semiovale PVS require external validation, ideally in a larger independent cohort.

Perivascular spaces are a key route for the drainage of interstitial fluid and solutes (including soluble amyloid-β) from the brain. Progressive amyloid-β deposition in small cortical and leptomeningeal arteries in CAA could gradually impair perivascular drainage,

causing retrograde dilation of perivascular spaces in the underlying white matter of the centrum semiovale, either by blocking bulk flow, or by diminishing the pulsatility of small vessels (required for efficient interstitial fluid drainage) due to smooth muscle cell loss.[263, 279] Impaired perivascular drainage could then further exacerbate vascular and perivascular amyloid-β deposition creating a "feed-forward loop".[274, 276] Consistent with this hypothesis, a post-mortem study of AD found that amyloid-β blocks perivascular drainage pathways in CAA associated with AD, and the degree of enlargement of white matter perivascular spaces correlates with CAA severity.[132] Moreover, a recent MRI study in ICH patients reported that very severe centrum semiovale PVS were associated with CAA defined according to clinical-radiological criteria.[272] However, more studies are needed to definitively determine the pathological basis of MRI-visible PVS.

We found no increased prevalence of basal ganglia PVS in CAA, consistent with deep perforating arteries being largely spared by amyloid-β deposition.[37] Previous studies report that neuroimaging markers of hypertensive arteriopathy (lacunes, deep cerebral microbleeds and white matter changes) are more strongly linked to basal ganglia than centrum semiovale PVS, also suggesting that the distribution of PVS may reflect the underlying arteriopathy.[30, 272, 274, 275] Since lobar ICHs are more likely to be surgically evacuated, our sample of non-CAA ICH is biased towards lobar ICH, which may contribute to the lack of severe basal ganglia PVS in the non-CAA group. However, non-CAA ICH cases more often had milder (1-10) centrum semiovale PVS compared to CAA cases.

Limitations of our study include the small cohort and selection bias due to the requirement for T2-weighted MRI. Because we included routinely collected haematoma evacuation samples, which may be suboptimal in comparison to post-mortem examinations, it was not possible to definitively assess in all patients whether vessels were parenchymal or leptomeningeal. Due to limited tissue sampling it is possible that some non-CAA ICH patients might have had undetected CAA. Moreover, the amount of available tissue (and number of assessable cortical and leptomeningeal vessels) was generally higher in the CAA group, which could have led to a higher chance of sampling error in the non-CAA group. However, misclassification and small sample size would tend to bias toward a null result. Although CAA patients were older than non-CAA patients, the difference in severe centrum semiovale PVS was robust to adjustment for age; furthermore, the CAA group had a higher prevalence of PVS only in the centrum semiovale, while confounding by age would be expected to increase PVS severity in both the centrum semiovale and basal ganglia.[270, 272, 274] Finally, although the PVS raters were blinded to clinical and histopathological information as well as microbleeds status, it was impossible to blind them to ICH presence.

Our findings are preliminary and hypothesis-generating, requiring external validation in larger independent cohorts with pathological verification of CAA. Nevertheless, if confirmed, PVS in the centrum semiovale may help improve the sensitivity of MRI for *in vivo* diagnosis of sporadic CAA in an appropriate clinical context. Further studies are also needed to determine whether centrum semiovale PVS precede other imaging features of CAA[280], which might help identify CAA earlier in its natural history.

Chapter 7 MRI-visible white matter perivascular spaces: a marker of cerebrovascular amyloid burden?

ABSTRACT

Objective: To investigate the relationship between MRI-visible centrum semiovale perivascular spaces (CSO-PVS), a biomarker of impaired interstitial fluid drainage, and PET-based amyloid-β burden across a wide range of cerebrovascular amyloid deposition, we analyzed a sample merging healthy subjects of variable age, a fraction of whom is expected to harbor significant cerebrovascular amyloid, and patients diagnosed with probable cerebral amyloid angiopathy (CAA).

Methods: Thirty-one non-demented subjects (11 patients with probable CAA, 10 age-matched healthy subjects >60yrs, and 10 healthy subjects <60yrs), had brain MRI and PiB-PET. CSO-PVS was evaluated on axial T2-weighted sequences using a validated 4-point scale and further classified as high (score>3) or low (score≤3) degree for pre-specified analyses. Linear regression was used to assess the association between whole cortex PiB and CSO-PVS.

Results: In multivariable linear regression adjusting for age, white matter changes and microbleeds count, whole cortex PiB binding was associated with CSO-PVS degree both as continuous variable (coefficient: 0.11; CI: 0.01-0.22, P=0.040) and as dichotomous variable (coefficient: 0.27; 95% CI: 0.11-0.44, p=0.002). In addition, the median PiB retention was higher in high compared to low CSO-PVS degree (median1.42; IQR: 1.37-1.50 vs. 1.14; IQR: 1.12-1.23, p=0.0007), an association also present within the healthy subjects group separately (p=0.023). All results remained consistent in sensitivity analyses excluding healthy subjects <60 years.

Conclusion: These results suggest a strong association between PiB uptake and CSO-PVS, which has important pathophysiological implications for both cerebrovascular amyloid deposition and CSO-PVS, but also highlights the possible utility of the latter as a surrogate for cerebrovascular amyloid burden.

7.1 Introduction

Perivascular (or Virchow-Robin) spaces are interstitial fluid-filled cavities surrounding small penetrating vessels,[281] which function to allow interstitial fluid and solute efflux from the brain.[129, 263, 276] Accumulating evidence suggests that MRI-visible perivascular spaces in the centrum semiovale (CSO-PVS) and basal ganglia (BG-PVS) are potential neuroimaging markers of cerebral small vessel disease.[30, 31, 272-275] PVS are also very common in healthy elderly subjects,[264, 270] in whom CSO-PVS are a risk factor for dementia.[271] CSO-PVS on MRI are also attracting increasing attention as a promising indicator of cerebrovascular amyloid deposition, a common age-related neuropathological process characterised by progressive amyloid-β accumulation within the walls of leptomeningeal and cortical small arteries, arterioles and occasionally capillaries.[28, 37]

Recent studies emphasize not only that cerebrovascular amyloid deposition is widely prevalent in the aged population, affecting up to 30-40% of healthy elderly,[58, 174] but also that it has become a major cause of spontaneous lobar intracerebral haemorrhage (ICH)[28, 37], can also cause or contribute significantly to cognitive impairment,[58, 155] and is almost invariably present in Alzheimer's disease.[65] CSO-PVS is important to consider in this context as it might reflect impaired interstitial fluid drainage from the white matter to the cortical surface and in turn contribute to cerebrovascular amyloid deposition.[276] Indeed, emerging evidence suggests that cerebrovascular amyloid deposition is a "protein elimination failure angiopathy":[282] as perivascular drainage pathways fail with age (or are overloaded by reduced capacity of other elimination mechanisms), amyloid-β is increasingly trapped in the perivascular compartment and deposited in the walls of small arteries.[276] However, the precise relationship and mechanisms between MRI-visible CSO-PVS and cerebrovascular amyloid deposition has not been explored *in vivo*.

^{11}C-Pittsburgh compound B (PiB) has been developed as a positron emission tomography (PET) radioligand for imaging both parenchymal and vascular fibrillar amyloid-β deposits, and has revolutionized research into potential pathomechanisms of cerebrovascular amyloid deposition and Alzheimer's disease.[48-50, 283-285] If MRI-visible CSO-PVS and perivascular amyloid-β deposition are indeed linked, one would expect cortical PiB retention to be associated with the overall CSO-PVS burden. If confirmed, this would in turn further strengthen the potential of CSO-PVS as a surrogate for vascular amyloid burden. To investigate this relationship across as wide a range of cerebrovascular amyloid burden and CSO-PVS as possible, we analysed in an MRI-PET pilot study a well-sized sample purposely merging patients diagnosed with probable cerebral amyloid angiopathy (CAA)

and healthy subjects across the adult age span, a fraction of whom is expected based on established neuropathological knowledge[58] to harbour some degree of both cerebrovascular amyloid and CSO-PVS.[264, 270]

7.2 Subjects and Methods

7.2.1 Study subjects

Non-demented lobar ICH patients fulfilling the original Boston criteria for probable CAA[195] were recruited through the Addenbrooke's Hospital Stroke Unit or ICH clinic. Consecutive eligible patients were approached to take part in the study. Admission records and clinic files were screened for discharged potentially eligible patients who were then contacted. All included CAA patients lived independently and their Mini-mental State Examination (MMSE) was >23 in all. Age at PiB PET, gender and presence of vascular risk factors were ascertained by medical records review and by patient and caregiver interview.

Medication-free healthy subjects with no memory or cognitive complaints and normal MMSE results (≥29) were also enrolled through advertisements posted at the University of Third Age and hospital inboard. The absence of memory or cognitive complaint was specifically verified, and to avoid any recruitment bias the participants were informed that they would not be given any results from their scans (MR or PET) unless an unexpected lesion was found. The healthy sample comprised both a group of healthy elderly individuals ≥60 years age-matched to the CAA group and a group of healthy adults <60 years of age.

All subjects underwent a brain ^{11}C-PiB PET scan and structural MRI on the same day.

7.2.2 PET

The methodology used for ^{11}C-PiB production and PET scanning in our centre has previously been described.[286] Briefly, PET scanning was performed on a GE Advance PET scanner. Following a ^{68}Ge/^{68}Ga transmission scan (15 minutes) for attenuation correction, ~550 MBq of ^{11}C-PiB was injected as a bolus through an antecubital vein, and the 90 min dynamic ^{11}C-PIB-PET scan was reconstructed using the PROMIS 3D filtered backprojection algorithm.[287]

The PET data analysis was completely automated and carried out by a physicist blinded to the results of the MRI (YTH). To facilitate segmentation, spatial normalization and delineation of anatomical regions of interest (ROIs), participants also underwent a

structural T1-weighted MRI scan on a 3T Siemens Tim-Trio (Siemens Medical Solutions, Erlangen, Germany), using a 3-dimensional MPRAGE scan (magnetization-prepared rapid gradient-echo sequence; repetition time/echo time/inversion time=2300 milliseconds/2.98 milliseconds/900 milliseconds; flip angle, 9°; 1 average; 176 slices; 256×256 matrix size; 1×1×1 mm voxel size). To allow the use of standard space ROIs, the MRI, and thence the co-registered PET images, was spatially normalised to the MNI/ICBM152 T1-weighted template using the symmetric image normalisation method (SyN).[288]

As previously described,[289] ROIs were defined on these spatially normalized images as the intersection between ≥65% thresholded grey matter segments and Automated Anatomic Labeling (AAL) ROIs. ROIs overlapping with areas affected by the ICH were excluded *a priori*. In order to mitigate the partial volume effects of cortical atrophy, the CSF probability map for each subject, smoothed to match the PET spatial resolution, was used to correct each voxel time-activity curve (TAC) for CSF fraction (f_{CSF}) through division by ($1-f_{CSF}$).[290, 291] For each ROI, distribution volume ratio (DVR) was estimated from the mean CSF-corrected TAC using the reference tissue Logan graphical method fitted over the 35-90min post-injection interval, with the cerebellum as the reference tissue.[292] Whole cortex DVR was obtained through volume-weighted averaging of the cortical ROI DVR values across both hemispheres.

7.2.3 MRI data and analysis

On the same day as the PiB-PET study, each subject underwent detailed structural MRI at 3T (slice thickness: 4mm, slice gap: 5mm), which included T2-weighted, T1-weighted MP-RAGE, FLAIR and T2*-GRE, using standard sequences. MR images were reviewed blinded to clinical and PET data. PVS were defined and rated on axial T2-weighted MR images, according to STandards for ReportIng Vascular changes on nEuroimaging (STRIVE),[31] by a trained observer 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) and CSO.[30, 272, 293] The numbers refer to PVS on one side of the brain: after reviewing all relevant slices for the anatomical area being assessed, the slice and side with the highest number of PVS was recorded. In cases of large lobar ICH, centrum semiovale PVS were assessed contralateral to the index ICH lesion; an estimation of the closest category ipsilateral to the lesion was made, and the highest severity was recorded. Intra-rater reliability testing of the PVS scale using an independent dataset of ICH scans (n=30) showed an intra-rater Cohen's kappa of 0.91 for BG-PVS and 0.82 for CSO-PVS. White matter hyperintensities of presumed vascular origin were assessed using the age-related white matter changes (ARWMC)

scale.[294] CMBs were evaluated on T2*-GRE (echo time=20 ms) images according to the Microbleeds Anatomic Rating Scale (MARS) and published guidelines.[35, 238]

7.2.4 Standard Protocol Approvals, Registrations, and Patient Consents

The study received ethical approval by the Cambridgeshire Regional Ethics Committee and all participants provided signed informed consent.

7.2.5 Statistical analysis

We compared basic demographic, clinical and imaging characteristics of CAA patients versus healthy subjects and age-matched elderly healthy subjects using appropriate univariable tests: chi-square test or Fisher's exact test for categorical variables, and Mann-Whitney U test for continuous variables. The relationship between whole cortex DVR and CSO-PVS degree, both included as continuous variables, was explored across the whole sample using Kendall's non-parametric correlation analysis. Linear regression analyses were then used to quantify the relationship between PiB DVR and CSO-PVS grade after adjustment for other biologically plausible covariates found to be related to both PiB retention and PVS in previous studies, including age, white matter hyperintensities total score and lobar CMBs count.[284, 295] We repeated the regression analysis using the CSO-PVS degree dichotomised into high (score >3) or low (score ≤3) (i.e. >40 CSO-PVS vs. ≤40 CSO-PVS respectively). This definition was pre-specified and is in line with the most severe category of CSO-PVS which might be more characteristic and sensitive for CAA.[270] This cut-off separated our data into two balanced groups. As a sensitivity analysis, we repeated the above linear regression models excluding healthy subjects <60 years (i.e. using only probable CAA patients and age-matched healthy elderly cases).

We also compared PiB retention (i.e. whole cortex DVR) between subjects with high vs. low CSO-PVS degree within the whole study population, and then separately within the CAA patients and the healthy group.

All tests of significance were 2-tailed, with values below the 10% level considered indicative of a trend. Statistical analyses were carried out using STATA (Version 12.1, StataCorp.).

7.3 Results

Eleven eligible CAA patients and 20 healthy subjects (ten elderly individuals ≥60 years and ten adults <60 years) were recruited and selected for the study (Table 7.1). One young healthy subject was excluded from the analysis *post-hoc* because of lack of adequate MRI sequences. All CAA patients presented with symptomatic lobar ICH (four patients had two symptomatic ICHs and one had three). ^{11}C-PiB-PET was performed within 3 years of first symptomatic lobar ICH. Two CAA patients had evidence of new asymptomatic lobar ICH on follow-up MRI.

PiB retention was higher in CAA patients compared to the whole healthy group (p=0.0082; Table 7.1), but similar between CAA patients and age-matched healthy subjects (p=0.53). High (>40) CSO-PVS degree was more common in CAA patients compared with the healthy group (p=0.003) and the age-matched elderly healthy subgroup (63.6% vs. 20%; p=0.08).

Table 7.1 Basic demographic and imaging characteristics of the study population. The p-values refer to the comparison between probable cerebral amyloid angiopathy cases (CAA) and age-matched elderly healthy subjects

Characteristics	Healthy subjects (n=20)	Probable CAA patients (n=11)	Age-matched elderly healthy subjects (n=10)	P-value
Age, median (IQR), years	59.5 (34.5-63.5)	71 (63-77)	63.5 (61-68)	0.06
Sex, male (%)	5 (50)	9 (81.8)	5 (50)	0.18
MMSE, median (IQR)	30 (29-30)	26 (25-28)	30 (29-30)	0.003
Hypertension, n (%)*	0	6 (54.6)	0	0.012
Lobar CMB presence, n (%)	1 (5)*	11 (100)	1 (10)*	<0.0001
Lobar CMBs count, median (IQR)	0	4 (2-50)	0	0.0002
Total WMH score, median (IQR)	2.5 (0-4)	11 (9-14)	4 (4-6)	0.0002
High degree CSO-PVS, n (%)	2 (10)	7 (63.6)	2 (20)	0.08
Whole cortex DVR, median (IQR)	1.14 (1.11-1.21)	1.37 (1.23-1.43)	1.21 (1.13-1.65)	0.53

* A healthy elderly subject had 3 lobar CMBs.

Figure 1 is the scatter plot of the relationship between whole-cortex PiB DVR and CSO-PVS degree across the whole cohort, showing a clear, nearly linear increase in PiB DVR as CSO-PVS increases, which was significant using non-parametric Kendall's correlation (tau-b = +0.552; p=0.0001). There was a trend for a correlation when only probable CAA patients and age-matched healthy elderly were included in the analysis (Kendall's tau-b=0.3289; p=0.068). In linear regression, PiB binding was positively associated with CSO-PVS degree both in the whole cohort and in sensitivity analyses, even after adjusting for age, CMBs and total white matter hyperintensities score (Table 7.2 and Table 7.3). There was a mean 0.11 (0.01-0.22) higher PiB retention for each increase in CSO-PVS degree (p=0.04) and 0.27 (95% CI: 0.11 to 0.44) higher PiB retention for CSO-PVS degree >40 versus ≤40 (p=0.002). This relationship was still significant in just the healthy group alone, and remained so when excluding the subject with CMBs.

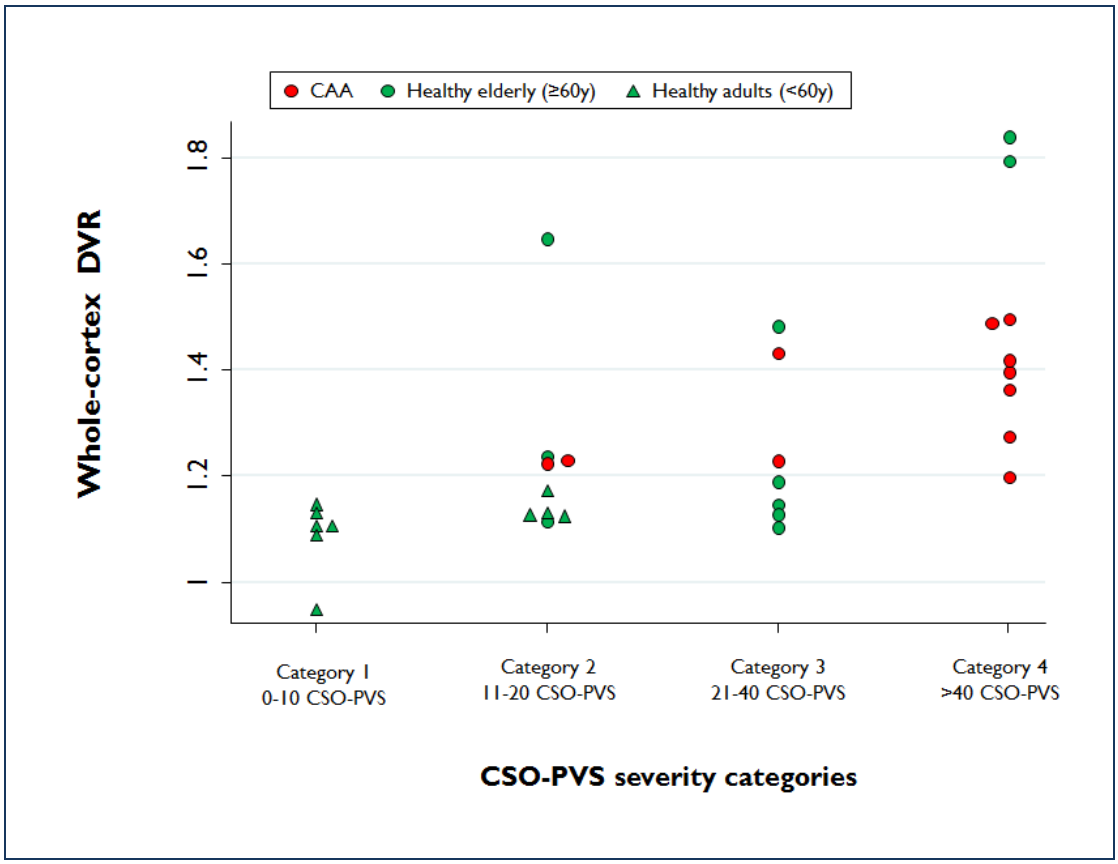

Figure 7.1 Scatterplot of whole cortex PiB retention (i.e. DVR) and centrum semiovale perivascular spaces (CSO-PVS) severity categories in cerebral amyloid angiopathy (CAA) healthy subjects. There was a positive correlation between PiB retention and increasing CSO-PVS categories across the whole group (Kendall's tau-b=0.552; p=0.0001).

Table 7.2 Univariable and multivariable linear regression analysis of the association between whole cortex PiB retention (DVR) and CSO-PVS grade (as continuous variable) in the whole study population and in sensitivity analyses including only probable CAA patients and the age-matched healthy elderly (≥60 years) subgroup.

Whole cohort	**PiB retention**	
	Coefficient (95% CI)	**p-value**
CSO-PVS, unadjusted	0.12 (0.06-0.18)	<0.0001
CSO-PVS, adjusted for age, CMBs and WMH	0.11 (0.01-0.22)	0.040
Probable CAA + age-matched healthy elderly		
CSO-PVS, unadjusted	0.11 (-0.01-0.22)	0.071
CSO-PVS, adjusted for CMBs and WMH	0.13 (-0.01-0.26)	0.059

CMBs: cerebral microbleeds; CSO-PVS: centrum semiovale perivascular spaces; WMH: White matter hyperintensities

Table 7.3 Univariable and multivariable linear regression analysis of the association between whole cortex PiB retention (DVR) and high CSO-PVS degree (>40) in the whole study population and in sensitivity analyses including only probable CAA patients and the age-matched healthy elderly (≥60 years) subgroup.

Whole cohort	**PiB retention**	
	Coefficient (95% CI)	**p-value**
CSO-PVS, unadjusted	0.28 (0.14-0.42)	<0.0001
CSO-PVS, adjusted for age, CMBs and WMH	0.27 (0.11-0.44)	0.002
Probable CAA + age-matched healthy elderly		
CSO-PVS, unadjusted	0.21 (0.04-0.39)	0.021
CSO-PVS, adjusted for CMBs and WMH	0.29 (0.08-0.49)	0.009

Across the whole cohort, whole cortex DVR was higher in subjects with a high CSO-PVS degree compared to low CSO-PVS degree (median1.42; IQR: 1.37-1.50 vs. 1.14; 1.12-1.23, respectively; p=0.0007). This was also true within the healthy group (1.82; 1.80-1.84 vs. 1.13; 1.11-1.18, respectively; p=0.023) as well as the healthy elderly (≥60 years) subgroup (1.82; 1.80-1.84 vs. 1.17; 1.12-1.36, respectively; p=0.037). The same trend was observed in the CAA group. There was no significant association between BG-PVS and PiB retention (data not shown).

7.4 Discussion

The present study is the first to address the relationship between cerebrovascular amyloid burden and CSO-PVS. Our data suggest a strong association between whole cortex amyloid burden and MRI-visible CSO-PVS in a combined cohort of patients diagnosed with probable CAA-related ICH and healthy elderly subjects without any memory or cognitive complaint, expected to represent a continuum of the degree of neuropathological vascular amyloid deposition.[58, 174] Furthermore, this association remained strongly significant after adjusting for potential covariates that may affect the severity of CSO-PVS and PiB binding, including age, WMH and CMB count. Importantly, the link between PiB binding and CSO-PVS was also present within the healthy elderly group alone, and showed similar trends in the CAA group. Finally, this association was not present with BG-PVS, i.e., it was specific for CSO-PVS, as it.

The design of the present study including in the same analysis both probable CAA-related lobar ICH patients and healthy controls was based on the strong neuropathological and *in vivo* MR evidence detailed in the Introduction that both cerebrovascular amyloid and CSO-PVS are present in the healthy elderly and form a continuum with symptomatic CAA. This also underlies the rationale for not excluding from this analysis the healthy subject who had 3 lobar CMBs and might therefore have incipient CAA. In order to test our hypothesis that CSO-PVS and cerebrovascular amyloid deposition are linked, we used PiB PET. Several lines of evidence have established that PiB PET detects not only parenchymal but also vascular amyloid-β deposits. Thus, radiologic-pathologic correlation studies showed that PiB binds to vascular amyloid.[201, 296] Moreover, increased PiB retention is spatially related to lobar CMBs in CAA[176] and may predict sites of future haemorrhage.[285] Significant amyloid burden on PET imaging is found in 20-40% healthy elderly individuals after age 60, despite normal cognition.[297, 298] Given the high prevalence of both Alzheimer's disease pathology and incipient CAA, increased PiB burden in healthy aged people is

thought to reflect not only very early Alzheimer's disease-related parenchymal accumulation of amyloid-β but also underlying asymptomatic cerebrovascular amyloid.[58, 174] Accordingly, in a recent study in asymptomatic older adults, PiB uptake was strongly associated with lobar CMBs, a putative marker of CAA, independent from APOE ε4 status, vascular risk factors, and antiplatelet therapy.[295] All these factors may explain why in the present sample there was no significant difference in whole cortex PiB retention or regional ratios between CAA patients and healthy subjects (see Baron et al[289] for further details and a discussion on this). The rationale of the present study, namely a continuum in cerebrovascular amyloid across healthy participants and probable CAA patients, was strongly supported by the significant relationship between CSO-PVS degree and PiB uptake found within the healthy control group alone. In other words, the significant findings in the merged sample do not merely reflect the higher CSO-PVS burden in the CAA subset.

Although the mechanisms of MRI-visible CSO-PVS remain poorly understood, our current findings highlight a potential pathophysiologic link between amyloid burden and CSO-PVS. This link may reflect interstitial fluid drainage impairment within the perivascular spaces by progressive vascular amyloid-β deposition, causing retrograde dilation of perivascular spaces in the underlying white matter of the centrum semiovale.[263, 279] Impaired perivascular drainage could then further exacerbate vascular and perivascular amyloid-β deposition in small cortical and leptomeningeal vessels, setting in motion a "feed-forward loop", by which cumulative amyloid deposition promotes further cerebrovascular amyloid deposition.[274, 276, 299] Consistent with this hypothesis, a post-mortem study of Alzheimer's disease found that amyloid-β blocks perivascular drainage pathways in cerebrovascular amyloid deposition associated with Alzheimer's disease pathology; the degree of white matter perivascular spaces enlargement correlated with cerebrovascular amyloid burden.[132] Moreover, a recent MRI study in spontaneous ICH patients reported that high grade CSO-PVS were strongly associated with CAA defined according to clinical-radiological criteria.[272] Similar associations were reported among subjects from a memory clinic, in which CSO-PVS was associated with cerebrovascular amyloid markers, whereas BG-PVS were indicative of hypertensive arteriopathy.[274] Our findings are also consistent with the hypothesis of BG-PVS have different pathophysiology to CSO-PVS and deep perforating arteries being largely spared by amyloid-β deposition.[37]

Strong points of our study include the systematic MRI evaluation for a range of imaging markers of cerebral small vessel disease, which allowed testing a pre-specified hypothesis, and the fact that MR was obtained on the same day as PET. The main limitation, intrinsic to amyloid imaging, is that incipient Alzheimer's disease pathology might also be present in non-demented ICH patients suspected of CAA, particularly given the frequent co-

occurrence and overlapping molecular mechanisms of these two amyloid-related conditions, but also in healthy elderly. Another limitation is the relatively small sample size limiting statistical power. For this reason our study should be considered preliminary and the p-values should be interpreted cautiously, particularly within the subgroups. As our study was cross-sectional, we were unable to determine the cause–effect relationship between increased PiB retention and CSO-PVS.

Although preliminary, our results suggest that amyloid-β burden in associated with CSO-PVS degree, and have implications for understanding cerebrovascular amyloid pathophysiology. Furthermore, our work further supports the idea of CSO-PVS being a useful marker of vascular amyloid burden in future studies of both clinically healthy subjects and symptomatic CAA cohorts, as well as disease-modifying trials.[300] Larger prospective cohorts are needed to validate our findings and clarify exactly how exactly CSO-PVS are related to amyloid deposition, impaired interstitial fluid drainage as well as other markers of small vessels disease in CAA.

Chapter 8 White matter perivascular spaces on MRI are related to cortical superficial siderosis in sporadic cerebral amyloid angiopathy

ABSTRACT

Objectives: We set out to investigate whether MRI-visible centrum semiovale perivascular spaces (CSO-PVS) - a potential biomarker of impaired interstitial fluid drainage in sporadic cerebral amyloid angiopathy (CAA) - is associated with cortical superficial siderosis (cSS), reflecting recurrent haemorrhage from severe leptomeningeal and superficial cortical vascular amyloid.

Methods: Retrospective multicentre cohort study of possible/probable CAA according to the Boston criteria. PVS were rated in basal ganglia (BG-PVS) and CSO (CSO-PVS) on axial T2-weighted sequences, using a validated 4-point visual rating scale, and were classified as high (score >2) or low degree (score ≤2) for pre-specified analyses. Independent risk factors for high CSO-PVS degree were investigated in logistic regression.

Results: The final cohort consisted of 138 CAA patients (mean age: 71.8; 95%CI: 70.2-73.4 years, 52.2% male). High CSO-PVS degree was present in 61.2% of cases. The prevalence of any cSS, and disseminated cSS (involving >3 sulci), was higher in patients with high vs. low CSO-PVS degree (for any cSS 45.9% vs. 13.5%; p<0.00005; for disseminated cSS 31.8% vs. 0%; p<0.00005). In multivariable logistic regression analysis, cSS presence (OR: 4.78; 95%CI: 1.64-13.87; p=0.004) was an independent predictors of high CSO-PVS degree. We found no associations between BG-PVS and cSS.

Conclusions: High degree of CSO-PVS is highly prevalent in sporadic CAA, and is related to cSS. Our findings suggest that severe leptomeningeal and cortical vascular amyloid (causing cSS) is related to impaired interstitial fluid drainage from cerebral white matter, although determining the causal direction of this relationship requires prospective studies.

8.1 Introduction

Sporadic cerebral amyloid angiopathy (CAA) is a common small vessel disease that results from progressive amyloid–β deposition within the walls of small cortical and leptomeningeal arteries.[28, 242] CAA is most often recognised in life by symptomatic, lobar intracerebral haemorrhage (ICH) and cognitive impairment in elderly patients.[28, 37, 242] CAA is also associated with characteristic magnetic resonance imaging (MRI) markers including strictly lobar cerebral microbleeds (CMBs) and white matter hyperintensities.[28, 35] These imaging markers might be suggestive of CAA even in asymptomatic elderly individuals.

Accumulating evidence suggests that cortical superficial siderosis (cSS)[171, 191, 194, 195] and MRI-visible centrum semiovale (i.e. cerebral hemisphere white matter) perivascular spaces (CSO-PVS) are new imaging markers of CAA, which may reflect distinct but related aspects of pathophysiology. However, the precise relationship between CSO-PVS and other imaging manifestations of CAA, including cSS and lobar CMBs has not been explored.

We hypothesised that progressive amyloid-β deposition in small leptomeningeal or superficial cortical vessels (leading to fragility, repeated haemorrhage into the subarachnoid space and cSS) blocks perivascular drainage pathways from white matter to the cortical surface, resulting in CSO-PVS. To test this hypothesis and to gain insights into potential mechanisms we investigated the relationship between MRI-visible CSO-PVS and cSS in patients with possible or probable CAA according to the Boston criteria.

8.2 Methods

8.2.1 Study population and data collection

Consecutive CAA patients(according to the original Boston criteria[202] – i.e. not including cSS as a criterion) from an ongoing multicentre cohort study at four stroke centres over defined time periods as previously described,[301, 302] were evaluated. The centres were: University College London Hospitals NHS Foundation Trust (London) (03/2003–09/2011), Addenbrooke's Hospital (Cambridge) (07/2002–03/2010), Cliniques Universitaires Saint Luc (Brussels) (12/2003–04/2010) and CHU Mont-Godinne UCL (Brussels) (08/2005–03/2009). At participating centres, MRI is a routine investigation for cases of suspected CAA, unless there are contra-indications. Essential inclusion criteria for the multicentre CAA cohort[301, 302] and this analysis were: (1) CAA, defined according to the Boston criteria[202] – including lobar CMBs, but not cSS; and (2) available MRI sequences of adequate quality including T2-

weighted, T2*-weighted gradient-recalled echo (T2*-GRE) and FLAIR MRI sequences. The selection process of this multicentre cohort has been previously described:[10, 11] in brief, from a total of 358 patients with suspected CAA/spontaneous ICH screened, 144 were initially excluded because of MRI was not performed or was not available/interpretable. After reviewing available MRI and clinical data, 56 patients not fulfilling the Boston criteria for CAA were further excluded, leaving a total of 158 CAA patients potentially eligible for the current analysis:[302] 59 patients from University College London Hospitals, 49 from Addenbrooke's Hospital, 36 from Cliniques Universitaires Saint Luc and 14 patients from CHU Mont-Godinne UCL. Patients with no T2 MRI (n=14), T2 MRI of insufficient quality (n=1) and irretrievable sequences (n=5) where further excluded from this analysis.

Demographic and clinical information was obtained from prospective databases and by medical records review using standardised data collection forms, as previously described.[301, 302] Hypertension was defined as a history of hypertension, taking antihypertensive treatment or documented elevated blood pressure (systolic >150 or diastolic >95mmHg) before admission, diabetes as ongoing use of a hypoglycaemic agent and smoking as history of tobacco use before admission.

The study received ethical approval by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee, the Commission d'Ethique Biomedicale Hospitalo Facultaire of the Faculte de Medicine (Université Catholique de Louvain), and the Comite d'ethique medicale of the CHU Dinant-Godinne (Université Catholique de Louvain).

8.2.2 Neuroimaging data and analysis

The MRI protocols were similar in each hospital. Imaging was performed at field strength of 1.5T for all patients and included T2-weighted (slice thickness: 4-6 mm, slice gap: 2-6 mm, echo time: 89-90/92 ms, relaxation time: 4408.8/6040/5610/6630/4520 ms), FLAIR, T2*-GRE (slice thickness 5 mm, slice gap: 1.5/5/6.5 mm, repetition time 500–1,000 ms, echo time 40/26/15/50-70 ms) and diffusion-weighted imaging (DWI) sequences. MR images were reviewed by a single trained rater blinded to clinical data.

PVS were assessed and rated on axial T2-weighted MR images, according to STandards for ReportIng Vascular changes on nEuroimaging (STRIVE),[31] by a trained observer 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) and CSO.[30, 267, 293] The numbers refer to PVS on one side of the brain: after reviewing all relevant slices for the anatomical area being assessed,

the slice and side with the highest number of PVS was recorded. The assessment of PVS may be influenced by the presence of confluent white matter hyperintensities; in such cases estimation was made of the closest PVS rating category, using the appearance of non-involved white matter, according to the rating scale used. In cases of large lobar ICH, centrum semiovale PVS were assessed contralateral to the index ICH lesion; an estimation of the closest category ipsilateral to the lesion was made, and the highest severity was recorded. Intra-rater reliability testing of the PVS scale using a dataset of ICH scans (n=30) showed an intra-rater Cohen's kappa of 0.91 for BG-PVS and 0.82 for CSO-PVS.

cSS was defined as linear residues of chronic blood products in the superficial layers of the cerebral cortex showing a characteristic "gyriform" pattern of low signal on T2*-GRE images, without corresponding hyperintense signal on T1-weighted or FLAIR images.[301] The distribution of cSS was classified as focal (restricted to ≤3 sulci) or disseminated (≥4 sulci).[195] The inter-rater agreement for the presence or absent of cSS in our group has been previously punished and was 89.6% (Cohen's kappa=0.79) and for cSS categories was 89.6% (weighted Cohen's kappa=0.75).[302] T2-weighted MR images were assessed for PVS blinded to cSS status on T2*-GRE and vice versa; both markers were rated blind to the study hypothesis.

White matter hyperintensities (leukoaraiosis) were assessed with the four-step simplified Fazekas rating scale, from 0 to 3 (0=no lesions; 1=focal lesions; 2=early confluent; 3=confluent).[239, 301] Lobar CMBs were evaluated on T2*-GRE images according to the Microbleeds Anatomic Rating Scale (MARS), as previously described.[35, 238, 301]

8.2.3 Statistical analysis

We pre-specified a dichotomised classification of PVS degree as high (score >2) or low (score ≤2). This definition is in line with the most severe category of CSO-PVS used in a previous study (and found to relate to vascular risk factors),[270] and appears to be sensitive for CAA diagnosis.[293] We compared demographic, clinical and imaging characteristics of CAA patients with high *versus* low CSO-PVS degree using appropriate univariable tests: chi-square test or Fisher's exact test for categorical variables, and Student t-test for age. Multivariable logistic regression analysis was used to explore the relation between high CSO-PVS degree and cSS adjusted for other variables based on the results of univariable analysis, plus other biologically plausible confounders related to PVS[264, 270, 272, 274, 303] or CAA severity, including age, sex, hypertension, WMH, CMBs number, and previous history of symptomatic ICH (see Results). We further adjusted our multivariable model for possible or probable CAA diagnostic category. We repeated these analyses only within the probable

CAA Boston diagnostic category. Because CMBs number distribution in our cohort was not normally distributed, as a sensitivity analysis, we repeated our multivariable logistic regression analyses by including multiple (≥2) CMBs or CMBs categories (0, 2-4, 5-10 and >10 CMBs) into the models, which remained consistent. Multicollinearity was assessed by computing variance inflation factors (VIF) for all predictors and removing all variables with VIF>5. None of our included predictors in multivariable models had a VIF>5. Significance level was set at 0.05 for all analyses. Statistical analyses were carried out using STATA (Version 11.2, StataCorp.).

8.3 Results

The final cohort consisted of 138 patients: 32 patients with possible CAA, 97 with probable CAA and 9 patients with supportive pathology based on the Boston criteria (Table 8.1). Of these, 120 (87%) patients presented with symptomatic lobar ICH at baseline, 9 with transient focal neurological episodes, 3 with cognitive decline, 1 with acute convexity subarachnoid haemorrhage and 5 with ischaemic stroke. All patients had some degree of MRI-visible CSO-PVS; high CSO-PVS degree was present in 61.2% (n=85) of cases. Fifteen (10.9%) individuals had 1-10 CSO-PVS, 38 (27.5%) had 11-20 CSO-PVS, 50 (36.2%) had 21-40 CSO-PVS and 35 (25.4%) patients had ≥40 CSO-PVS. There was no correlation between BG-PVS and CSO-PVS severity (data not shown).

cSS was detected in 46 (33.3%; 95%CI: 25.5%-41.9%) CAA patients; 19 (13.8%; 95%CI: 8.5%-20.7%) patients had focal and 27 (19.7%; 95%CI: 13.3%-27.2%) had disseminated cSS. Patients with cSS more often had ≥5 CMBs than patients without siderosis (47.8% vs. 30%; p=0.040) and a higher (but not statistically significant at the 5% level) prevalence of small acute ischaemic lesions on DWI sequences (22% vs. 11.3%; p=0.117).

Comparisons of characteristics between patients with high degree and low degree of CSO-PVS are summarised in Table 8.1. Representative examples of cSS and CSO-PVS are shown in Figure 8.1. The prevalence of any cSS was higher in patients with high CSO-PVS degree (45.9% vs. 13.5%; p<0.00005). Disseminated cSS was present in 31.8% of participants with high CSO-PVS degree but none of those with low CSO-PVS degree (p<0.00005). High degree of CSO-PVS was associated with lower prevalence of moderate-to-severe leukoaraiosis and lower prevalence of hypertension (Table 8.1). cSS extent (i.e. no cSS, to focal and disseminated) was associated with CSO-PVS degree in ordinal logistic regression analysis (OR: 3.22; 95% CI: 1.99-5.21; p<0.00005). We found no association between BG-PVS and cSS.

Table 8.1 Characteristics and comparison of CAA patients with and without severe centrum semiovale perivascular spaces (CSO-PVS). P-values refer to differences between CAA patients with vs. without CSO-PVS, using chi-square tests and the Fisher's exact test for categorical variables, and two-sample t-tests or Mann-Whitney U-tests depending on the distribution of continuous variables.

Characteristics	All CAA (n=138)	High degree of CSO-PVS (n=85)	Low degree of CSO-PVS (n=53)	p-value
Age, mean (95% CI:), years	71.8 (70.2-73.4)	72.6 (71-74.3)	70.5 (67.2-73.7)	0.189
Sex, male (%)	72 (52.2)	43 (50.6)	29 (54.7)	0.637
Hypertension (%)	80 (63)	39 (50.7)	41 (82)	<0.0001
On antithrombotics (%)	36 (27.5)	24 (29.6)	12 (24)	0.483
History of symptomatic ICH (%)	44 (32.8)	22 (26.8)	22 (42.3)	0.063
Acute ischaemic lesions (%)	18 (14.9)	14 (18)	4 (9.3)	0.287
Lobar CMB presence (%)	91 (66.9)	58 (68.2)	33 (64.7)	0.672
Lobar CMBs count, median (IQR range)	2 (0-8)	2 (0-9)	2 (0-6)	0.479
Presence ≥5 lobar CMBs (%)	49 (36)	35 (41.2)	14 (27.5)	0.107
cSS presence (%)	46 (33.6)	39 (45.9)	7 (13.5)	<0.00005
Focal cSS (%)	19 (13.9)	12 (14.1)	7 (13.5)	0.914
Disseminated cSS (%)	27 (19.7)	27 (31.8)	0 (0)	<0.00005
Moderate-to-severe white matter hyperintensities (Fazekas score 2-3) (%)	23 (16.9)	10 (11.8)	13 (25.5)	0.039

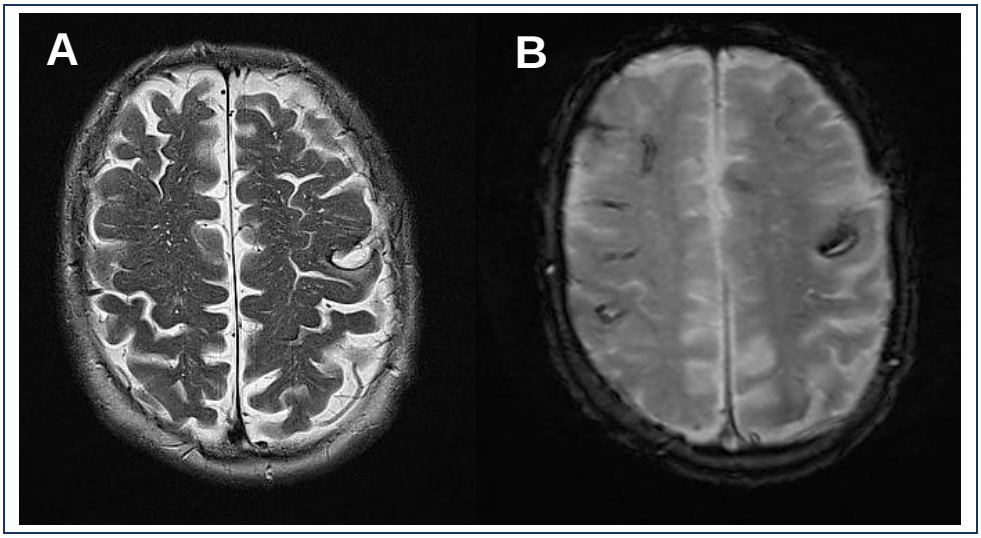

Figure 8.1 T2 and T2*-GRE MRI of a patient with probable cerebral amyloid angiopathy showing (A) High degree of CSO-PVS on MRI (small, sharply delineated structures of CSF intensity (or close to CSF intensity), following the course of cortical vessels), associated with (B) multifocal cortical superficial siderosis (cSS) especially in the right hemisphere.

In multivariable logistic regression models, high degree of PVS in the CSO was independently associated with the presence of cSS (OR: 4.78; 95%CI: 1.64-13.87; p=0.004) after adjusting for age, sex, hypertension, CMBs number, white matter hyperintensities, previous history of symptomatic ICH and probable vs. possible CAA. All results were of similar effect size in sensitivity analyses including only patients with probable CAA (Table 8.2). Both models remained consistent when multiple (≥2) CMBs or CMBs categories (0, 2-4, 5-10 and >10 CMBs) were included instead of CMBs number.

Table 8.2 Univariate (unadjusted) and multivariable (adjusted) logistic regression analysis of associations with high degree centum semiovale perivascular spaces (CSO-PVS), in the whole cohort of patients with cerebral amyloid angiopathy (CAA) and in patients with probable CAA. Both models remained consistent when multiple (≥2) CMBs or CMBs categories (0, 2-4, 5-10 and >10 CMBs) were included.

Whole CAA cohort (n=138)	**Unadjusted**		**Adjusted**	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
cSS presence	5.45 (2.21-13.45)	<0.0001	4.78 (1.64-13.87)	0.004
Age (per year increase)	1.03 (0.99-1.06)	0.189	1.02 (0.97-1.06)	0.501
Hypertension	0.23 (0.10-0.53)	0.001	0.27 (0.10-0.74)	0.011
Sex (male vs. female)	0.85 (0.43-1.69)	0.637	0.85 (0.33-2.18)	0.735
CMBs count	1.01 (0.98-1.04)	0.449	1.15 (0.54-2.42)	0.718
History of symptomatic ICH	0.50 (0.24-1.04)	0.065	0.65 (0.26-1.61)	0.354
WMH (moderate-to-severe)	1.10 (0.55-2.20)	0.709	1.78 (0.66-4.73)	0.254
Probable vs. possible CAA	1.58 (0.71-3.51)	0.263	0.69 (0.17-2.82)	0.608
Probable CAA (n=106)				
cSS presence	5.29 (2.05-13.65)	0.001	4.05 (1.36-12.08)	0.012
Age (per year increase)	1.04 (1.00-1.08)	0.073	1.03 (0.98-1.09)	0.235
Hypertension	0.24 (0.09-0.63)	0.004	0.28 (0.09-0.89)	0.032
Sex (male vs. female)	0.78 (0.35-7.74)	0.543	1.30 (0.43-4.00)	0.642
CMBs count	(0.98-1.04)	0.582	1.00 (0.97-1.04)	0.824
History of prior symptomatic ICH	0.63 (0.27-1.47)	0.288	0.83 (0.28-2.40)	0.724
WMH (moderate-to-severe)	0.90 (0.40-2.03)	0.799	1.18 (0.39-3.59)	0.768

8.4 Discussion

In this multicentre study we have shown that high degree of MRI-visible CSO-PVS are highly prevalent in patients with CAA and are related to the presence and severity of cSS, a characteristic neuroimaging marker of CAA.[195, 301] This association further adds to the accumulating evidence that CSO-PVS (but not BG-PVS) might be another potential MRI marker of CAA with implications for improving the sensitivity of *in vivo* diagnosis.[272, 274, 293] We found neither an association between BG-PVS and any CAA-specific imaging markers (including cSS and lobar CMBs), nor any correlation between severe CSO-PVS and BG-PVS, supporting the hypothesis that MRI-visible CSO-PVS compared to BG-PVS are due to different pathophysiological processes.[272, 274, 293] The structure of perivascular spaces in the basal ganglia, however, differs from those in the cerebral cortex, in that they are surrounded by two periarterial membranes, perhaps making them less vulnerable to vascular amyloid accumulation.[304] Our findings also have implications for understanding CAA pathophysiology, and suggest that severe leptomeningeal and cortical vascular amyloid (causing cSS) might be related to impaired interstitial fluid drainage from cerebral white matter (causing severe CSO-PVS).

The link between CSO-PVS and CAA may reflect interstitial fluid drainage impairment within the perivascular spaces caused by leptomeningeal and superficial cortical vascular amyloid-β - a major feature in sporadic CAA and Alzheimer's disease. Perivascular spaces form important pathways (along the basement membranes of capillaries and arteries) for the drainage of interstitial fluid and solutes, including soluble amyloid-β, from the brain. One key hypothesis for the development of CAA is that as perivascular drainage pathways fail with age (particularly in association with the ApoE e4 allele), or are overloaded by reduced capacity of other elimination mechanisms, amyloid-β is increasingly trapped and deposited in the walls of small arteries.[276] Amyloid-β deposition in small cortical and leptomeningeal arteries in CAA could disrupt drainage, leading to retrograde dilation of perivascular spaces in the underlying white matter of the centrum semiovale (either by blocking bulk flow, or by diminishing the pulsatility of small vessels required for efficient interstitial fluid drainage),[263, 279] leading to CSO-PVS visible on MRI. Cumulative superficial amyloid deposition could then promote further amyloid deposition, creating a "feed-forward loop", further reducing drainage efficiency.[274, 276, 299]

Of note, histopathological studies have shown a gradient of reducing vascular amyloid-β severity moving from the cortical surface into the cerebral white matter: CAA is significantly more severe in leptomeningeal than in parenchymal vessels of the same brain

section,[67] and superficial cortical layers have more extensive vascular amyloid-β deposition than deeper layers.[305] Repeated episodes of haemorrhage from these brittle, severely CAA-affected leptomeningeal or very superficial cortical vessels into the subarachnoid space are probably an important cause of cSS. Indeed, in CAA, cSS has a characteristic predilection for the cerebral convexities, reflecting linear blood residues in the superficial layers of the cerebral cortex or in the subarachnoid space.[190, 195, 243]

Our study has several strengths including the systematic evaluation of MRI scans by trained raters using validated scales for a range of imaging markers of small vessel disease and the testing of a prespecified hypothesis. The main limitation is the potential selection bias due to the requirement for MRI done as part of routine clinical care; hence, our results can only be extrapolated to patient populations in a similar clinical context with available MRI and need to be validated using larger independed cohorts. Also, although the MRI protocols were similar in each hospital, they varied across centres, introducing another potential source of bias. However, our findings are unlikely to be accounted by differences in MRI sequence parameters. Although we did our best to blind the MRI rating of cSS from CSO-PVS severity and vice versa (e.g. by assessing T2-weighted MR images for PVS blinded to cSS status on T2*-GRE and vice versa, by rating both markers blinded to the study main hypothesis etc.), we do acknowledge that the blinding might not be complete. In addition, the retrospective cross-sectional design prevents conclusions on the causal direction of the reported associations. Although it is likely that cSS and severe CSO-PVS each reflect the overall CAA severity in the brain, severe CSO-PVS was not associated with lobar CMBs number, a putative marker of CAA, indicating that the two phenomena may be causally related to each other, independed of CAA severity. In addition, we may have had insufficient statistical power to confirm or refute an association between CSO-PVS and small acute ischaemic lesions on DWI, due to our limited sample size and the relatively low prevalence of these lesions in CAA. However, severe CAA is associated with numerous cortical microinfarcts, which are difficult to resolve on 1.5T MRI, as used in this study. It may be of interest to study the relation of CSO-PVS and microinfarcts on high field strength in the future.[197] The inverse association between high CSO-PVS degree and presence of hypertension is also interesting and may reflect a protective effect related to aggressive treatment of hypertension in these patients. Finally, we did not have pathological confirmation of CAA, and acknowledge that the Boston criteria for CAA diagnosis have imperfect specificity, especially for the "possible CAA" category, which in some cases may include other cerebral small vessel diseases (e.g. hypertensive arteriopathy).[202, 203]Our study should be considered hypothesis-generating. Larger prospective CAA cohorts are needed to clarify how exactly CSO-PVS are linked to cSS (i.e. to determine the causal direction of

this relationship) as well as other specific MRI markers, and whether they might identify an imaging phenotype of CAA with more superficial disease. A key question for future work will be whether CSO-PVS are an early marker of a CAA-related disease process which ultimately leads to severe cortical and leptomeningeal amyloid deposition, with important implications for future haemorrhage risk.[302] Both cSS and PVS have been shown to be more prevalent in memory clinic patients (with Alzheimer's disease or mild cognitive impairment) compared to healthy controls;[243, 306-308] it would be very interesting to investigate whether patients with cSS and CSO-PVS differ from those without these findings with regard to cognitive function. MRI-visible PVS are also quite common in the general population, especially with increased age,[264, 303, 308, 309] and might be related with worse cognitive function[267] and incident dementia.[271]

Chapter 9 Cerebral amyloid angiopathy with and without intracerebral haemorrhage: MRI-pathological evidence for different disease phenotypes

ABSTRACT

Objective: To gain further insights into different cerebral amyloid angiopathy (CAA) phenotypes and mechanisms, we investigated cortical superficial siderosis (cSS) - a new imaging marker of the disease, and its relation with APOE genotype in patients with pathologically-proven CAA, who presented with and without intracerebral haemorrhage (ICH).

Methods: MRI scans of 105 consecutive patients with pathological confirmation of CAA and MRI obtained during life were analysed for cSS (focal, ≤3 sulci; disseminates, ≥4 sulci), and other small vessel disease markers. We compared pathological, imaging and APOE genotype data between subjects with vs. without ICH, and investigated associations between cSS and APOE genotype.

Results: Our cohort consisted of 54 CAA patients with symptomatic lobar ICH and 51 without ICH. APOE genotype was available in 53 patients. More than 90% of pathology samples in both groups had neuritic plaques, whereas neurofibrillary tangles were more commonly present in the non-ICH patients (87% vs. 42%, $p<0.0001$). On the other hand, the combination of neuritic plaques and neurofibrillary tangles was more common in the CAA group without ICH (84.8% vs. 41.7%, $p=0.001$). There was a trend for CAA patients with ICH to more commonly have APOE ε2 (48.7% vs. 21.4%, $p=0.075$), whereas non-ICH patients were more likely to be APOE ε4 carriers (85.7% vs. 53.9%, $p=0.035$). Disseminated cSS was considerably commoner in ICH patients (33.3% vs. 5.9%, $p<0.0001$). In logistic regression, disseminated cSS was associated with APOE ε2 (but not APOE ε4) (OR: 5.83; 95% CI: 1.49-22.82, $p=0.011$).

Conclusions: This neuropathological-defined cohort of CAA patients suggests that cSS and APOE ε2 are related to the hemorrhagic expression of the disease. In addition, APOE ε4 is enriched in non-hemorrhagic CAA; plaques and tangles pathology appears to be different in the two groups. Our study emphasizes that different clinical presentations of CAA have distinct features. This may suggest divergent pathophysiological mechanisms underlie these different CAA phenotypes.

9.1 Introduction

Sporadic cerebral amyloid angiopathy (CAA) is a small vessel disease that preferentially involves small cortical and leptomeningeal arteries due to progressive amyloid-β deposition in their walls.[28, 242] CAA occurs frequently in elderly people, and is now considered a common and important cause of symptomatic lobar intracerebral haemorrhage (ICH).[28, 37, 242] However, it has long been recognised that CAA might present without major lobar ICH but instead with cognitive impairment (either chronic or rapidly progressive), a very clinically salient manifestation of the disease, or transient focal neurological symptoms.[167] CAA is also almost invariably found in Alzheimer's disease, but in most cases is relatively mild.[65]

CAA is also associated with characteristic magnetic resonance imaging (MRI) biomarkers including strictly lobar cerebral microbleeds (CMBs), cortical superficial siderosis (cSS),[171, 191, 194, 195] centrum semiovale perivascular spaces (CSO-PVS) and white matter hyperintensities (WMH).[28, 35] Lobar CMBs might be suggestive of CAA even in asymptomatic elderly individuals.[174] These neuroimaging markers probably reflect related but distinct aspects of CAA pathophysiology. cSS in particular, is an interesting recently recognized form of CAA-related haemorrhage, which likely reflects repeated episodes of blood leaking into the subarachnoid space from brittle and fragile CAA-affected vessels. Recently, cSS has been shown to carry a high risk of future symptomatic lobar ICH.[195, 246, 302]

Genetic factors, such as apolipoprotein E (APOE) genotype, are important in the pathophysiology of CAA.[80, 84] APOE ε4 appears to enhance vascular amyloid-β deposition in a dose-dependent fashion,[310] whilst APOE ε2 promotes vasculopathic changes which can lead to vessel rupture.[88] To gain further insights into different CAA phenotypes and potential mechanisms,[311] we investigated associations between neuroimaging markers of the disease, APOE genotype, and pathologic findings in patients with CAA presenting with and without symptomatic ICH. We hypothesized that: (1) hemorrhagic markers of CAA (cSS and lobar CMBs) would be more strongly associated with APOE ε2 genotype; (2) hemorrhagic markers of disease severity in CAA (cSS and lobar CMBs) and APOE ε2 genotype would be more common in patients presenting with symptomatic ICH than in those presenting without ICH; and (3) neurofibrillary tangles will be more common in the non-haemorrhagic compared to haemorrhagic CAA, whereas amyloid plaques might be invariably present in the two groups due to their close molecular pathogenesis.

9.2 Methods

9.2.1 Case selection and clinical data collection

We included all eligible patients from the Massachusetts General Hospital (MGH) identified retrospectively by a systematic keyword search of pathology reports and prospective clinical databases. Cases were defined as subjects with both pathology-proven CAA (from routinely collected brain biopsy, biopsy at haematoma evacuation or autopsy) and adequate brain MRI sequences for the study. Data search covered patients seen at the hospital between 1997 and 2012. An additional overlapping search through established prospective datasets of patients with lobar haemorrhages (ICH and/or CMB) was performed to confirm identification of all potential eligible cases for the study. Among more than 3200 retrieved cases, we initially included those having: 1) a pathology report containing explicit information regarding CAA assessment; and 2) available brain MRI sequences of adequate quality including T2-weighted, T2*-weighted gradient-recalled echo (T2*-GRE) and/or SWI and FLAIR sequences. After reviewing all neuroimaging, pathological and clinical data available, we excluded subjects with: 1) small brain biopsy samples (greater diameter <1 cm) or samples from clot evacuations not containing any assessable vessels; 2) autopsy studies not grading CAA; and 3) known alternative causes for lobar ICH. A total of 192 patients were eligible for analysis. Patients with no pathological evidence of CAA (n=54), clinical/imaging presentations not characteristic of CAA (3 cases with deep ICH and 9 with ischaemic stroke at baseline), no T2 or T2*-GRE MRI (n=18), T2/T2*-GRE MRI of insufficient quality (n=2) and irretrievable sequences (n=1) were further excluded.

Demographic and clinical information was obtained from prospective databases and by medical records review using standardised data collection forms. Variables of interest were age, gender, history of hypertension, antithrombotic drug use and clinical presentation at baseline. Hypertension was defined as a history of hypertension, taking antihypertensive treatment or documented elevated blood pressure (systolic >150 or diastolic >95mmHg) before admission. APOE genotype was determined in a subset of patients who provided blood samples and consented to genetic testing as previously described,[312] and without knowledge of clinical or neuroimaging data.

The original clinical presentation of patients included in the study was ascertained from all available neuroimaging, pathological and clinical data, and was determined as either symptomatic lobar ICH confirmed on neuroimaging or non-haemorrhagic (including cognitive impairment, transient focal neurological episodes, or other neurological

symptoms). Cases of inflammatory CAA were also included in the analysis when an MRI outside the acute phase (≥1 month) was available. To investigate how stable these clinical phenotypes are (e.g. CAA with ICH and CAA without ICH), for the patient group presenting without ICH we extrapolated follow-up information from prospective databases and medical records on incident symptomatic ICH.

9.2.2 Standard Protocol Approvals, Registrations, and Patient Consents

The study received ethical approval by the Institutional Review Board of MGH.

9.2.3 Pathological data collection

Morphological assessment was performed in routine haematoxylin-eosin staining and the presence or absence and severity of vascular amyloid-β deposition was confirmed by immunohistochemical detection and/or congo red staining. CAA presence and severity was assessed in all available vessels. Cases were considered positive for CAA when they had at least one leptomeningeal or cortical vessel with amyloid-β reported, providing enough information to reliably classify CAA severity using the Vonsattel grading system[67, 72] and were classified as mild (Vonsattel grade 1) or moderate to severe (Vonsattel grades 2-4). Where available from neuropathology reports, we also systematically extracted information on: 1) neuritic plaques and neurofibrillary tangles assessed in routinely immunostained sections for amyloid-β and phosphorylated tau and recorded as present or absent; and 2) the presence or absence of moderate to severe arteriolosclerosis features (including thick arteriolar walls, lipohyalinosis, perivascular haemosiderin-laden macrophages etc.) mainly in the basal ganglia and deep cerebral white matter.[2]

9.2.4 Neuroimaging data and analysis

Imaging for all patients included T2-weighted, FLAIR, T2*-GRE and or susceptibility-weighted imaging (SWI). MR images were reviewed blinded to clinical, histopathological and genetic data by trained observers, according to STandards for ReportIng Vascular changes on nEuroimaging (STRIVE).[31]

CMBs presence and number were evaluated on axial T2*-GRE or SWI images according to current consensus criteria[35] and categorised as lobar (i.e. cortical-subcortical),

deep (i.e. basal ganglia, thalami, brainstem) or cerebellar. The presence and number of "macro" ICHs (>5 mm in diameter on T2*-GRE/SWI)[198] was also noted.

cSS was defined as linear residues of chronic blood products in the superficial layers of the cerebral cortex showing a characteristic "gyriform" pattern of low signal on T2*-GRE/SWI images; T1-weighted and FLAIR images were used for anatomical confirmation of the gyral location of these signal hypointensities identified on blood-sensitive sequences.[301] The distribution and severity of cSS was classified as focal (restricted to ≤3 sulci) or disseminated (≥4 sulci).[195] Areas of cSS were ≥2 unaffected sulci away from any lobar ICH, at multiple axial levels; cSS contiguous or potentially anatomically connected with any lobar ICH were not included in these categories.[301]

PVS were assessed in line with STRIVE definitions[31] and 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) and CSO as previously described.[30, 267, 293]

Periventricular and deep WMH were visually assessed on axial FLAIR images on the four-point Fazekas rating scale for each adding up to a total score on 7-point scale.[313] For deep WMH, 0 indicating absent; 1 indicating punctuate; 2 indicating early confluent; or 3 indicating confluent. For periventricular WMH, WMH 0 indicating caps; 1 indicating pencil-thin linings; 2 indicating smooth halos; or 3 indicating irregular appearance. Total WMH was dichotomized to score 0-4 vs. 5-6.

9.2.5 Statistical analysis

Categorical variables were analysed using Pearson's χ^2 or Fisher exact test, and continuous variables by the 2-sample t test (for normal distributions), and Wilcoxon rank sum (for non-normal distributions). We compared demographic, genetic, pathological and imaging characteristics of CAA patients with and without ICH using these univariate tests as appropriate. Variables for APOE ε2 and ε4 were each coded as the number of alleles per participant (0, 1, or 2). Separate logistic regression models were used to assess the relationship between APOE genotype and cSS (presence or burden), as well as CMB count (linear regression). As sensitivity analyses, these models were predetermined to adjust for age and clinical presentation of CAA. All tests of significance were two-tailed. Significance level was set at 0.05 for all analyses. Stata software (Version 11.2, StataCorp.) was used. The manuscript was prepared with reference to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines.[258]

9.3 Results

Our final cohort consisted of 105 patients with pathological evidence of CAA: 52 from autopsies, 22 from brain biopsies and 31 with pathological samples from haematoma evacuations. Fifty-four patients were admitted with symptomatic, spontaneous lobar ICH, while 51 patients without any symptomatic ICH. Patients without ICH presented either with cognitive impairment (n=42), transient focal neurological episodes (n=3), or a combination of other symptoms (n=6; including altered mental status, or seizures, with findings consistent with inflammatory CAA). The median Clinical Dementia Rating (CDR) score of patients without ICH and available data (n=27) was 1 (IQR: 0.5-2).

Table 9.1 indicates the severity of CAA, and the presence or absence of neuritic plaques and neurofibrillary tangles, in the two groups. In general, mild (Vonsattel grade 1) or moderate to severe (Vonsattel grades 2-4) CAA were equally represented in the cohorts ($p>0.2$ for both comparisons). There was no difference in the presence of vasculopathic changes (vessel-within-vessel appearance and vessel wall necrosis) in the two groups (29.6% vs. 24%, $p= 0.336$). Although the prevalence of neuritic plaques was very similar in the two groups, neuritic plaques in isolation (i.e. with no tangles) were much more frequent in the ICH patients than in the non-ICH CAA patients (53% vs. 13%, $p<0.0001$). More than 90% of pathology samples in both groups had neuritic plaques, whereas neurofibrillary tangles were more commonly present in the non-ICH patients (87% vs. 42%, $p<0.0001$). These associations remained consistent in logistic regression models controlling for age.

Comparisons of clinical and imaging characteristics between CAA patients with vs. without ICH are summarised in Table 2. There was a trend for CAA patients with ICH to more often have APOE ε2 (48.7% vs. 21.4%, $p=0.075$), whereas non-ICH patients were more likely to be carriers of APOE ε4 (85.7% vs. 53.9%, $p=0.035$). The two groups were very similar in imaging markers of small vessel disease, including WMH burden, high degree of CSO-PVS and BG-PVS and lobar CMBs counts (

Table 9.2). However, the prevalence of cSS was higher in patients with ICH (51.9% vs. 19.6%, $p=0.001$), especially disseminated cSS (33.3% vs. 5.9%, $p<0.0001$). Representative MR images are showing in Figure 9.1.

Table 9.1 Cerebral amyloid angiopathy with and without intracerebral haemorrhage: severity and associations with neuritic plaques (NP) and tangles (NT)*.

	CAA severity (Vonsattel grade)		Vasculopath changes	NP alone	NT alone	NP and NT	No NP or NT
	Grade 1	Grade 2-4					
CAA with ICH	7 (13%)	47 (87%)	16 (29.6%)	19 (52.8%)	0	15 (41.7%)	3 (5.6%)
CAA without ICH	11 (21.6%)	40 (78.4%)	12 (24%)	6 (13%)	1 (2.2%)	39 (84.8%)	0

*Data on neuritic plaques and tangles were available in 37/54 patients with ICH and 46/51 patients without ICH.

Table 9.2 Comparison of clinical, imaging and genetic characteristics between pathologically-proven CAA with vs. without ICH cohorts.

	Whole CAA cohort (N=105)	CAA with ICH (N=54)	CAA without ICH (N=51)	p-value
Age, mean (95% CI), years	72.7 (71.1-74.2)	71.6 (69.4-73.7)	73.9 (71.5-76.2)	0.148
Sex, female n (%)	55 (52.4)	33 (63.1)	22 (43.1)	0.065
Hypertension, n (%)	66 (64.7)	30 (57.8)	36 (72)	0.131
Antithrombotic drug use, n (%)	40 (44.4)	15 (36.6)	25 (51)	0.170
***APOE ε2 present, n (%)**	22 (41.5)	19 (48.7)	3 (21.4)	0.075
***APOE ε4 present, n (%)**	33 (62.3)	21 (53.9)	12 (85.7)	0.035
Time from MRI to pathology, median (IQR), months	5.3 (0.3-31.4)	1.3 (0.2-11)	23.3 (1.4-50.7)	0.0002
Premorbid diagnosis of Alzheimer's disease, n (%)	24 (22.9)	2 (3.7)	22 (43.1)	<0.0001
Severe (Fazekas 5-6) WMH, n (%)	21 (20)	13 (24.1)	8 (15.7)	0.283
High grade CSO-PVS (>20), n (%)	57 (54.8)	29 (54.7)	28 (54.9)	0.985
High grade BG-PVS (>20), n (%)	25 (24)	11 (20.8)	14 (27.5)	0.424
Lobar CMBs presence, n (%)	63 (60)	36 (66.7)	27 (52.9)	0.151
Lobar CMBs count, median (IQR)	2 (0-27)	3 (0-23)	2 (0-31)	0.411
Presence of cSS, n (%)	38 (39.2)	28 (51.9)	10 (19.6)	0.001
Focal cSS, n (%)	17 (16.2)	10 (18.5)	7 (13.7)	0.505
Disseminated cSS, n (%)	21 (20)	18 (33.3)	3 (5.9)	<0.0001

*APOE genotype was available in 53 CAA patients: 39 with ICH and 14 without ICH.

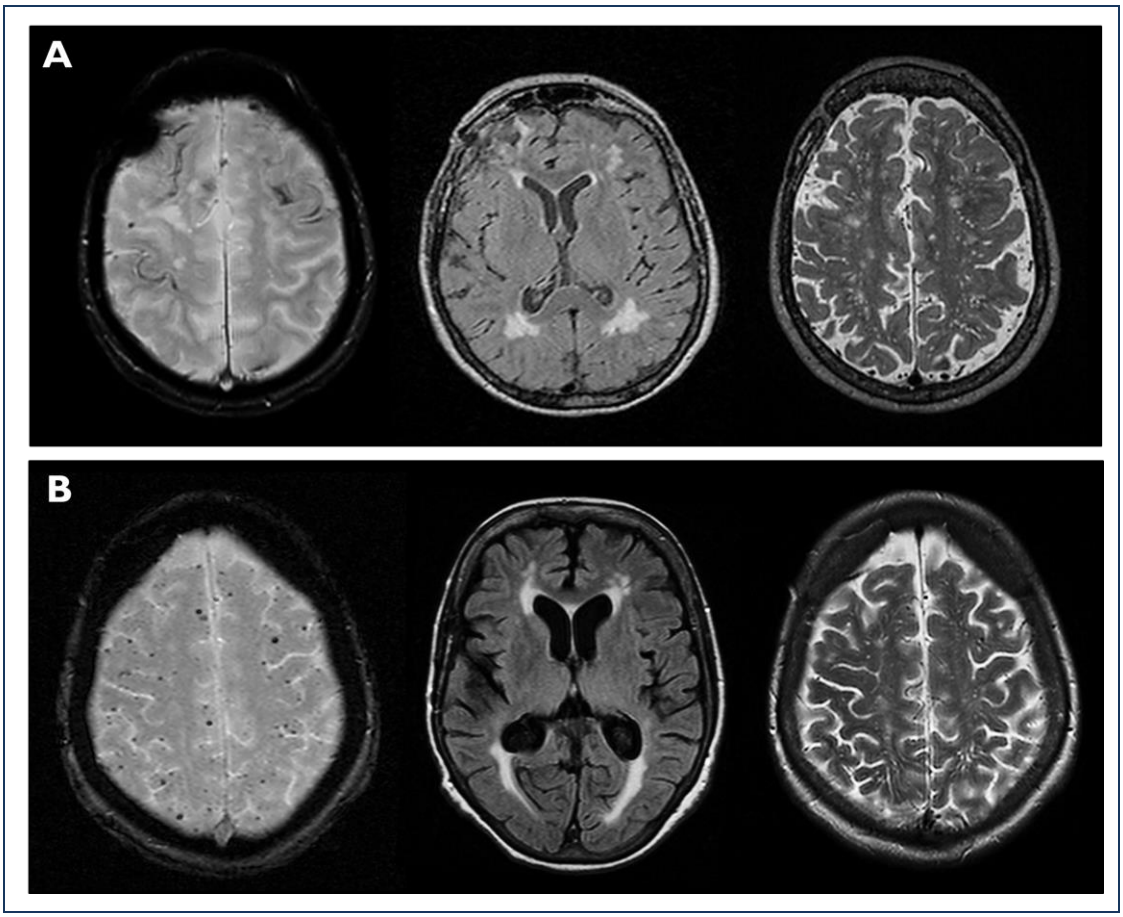

Figure 9.1 Representative MR images of patients with pathological evidence of cerebral amyloid angiopathy (CAA) with and without symptomatic intracerebral haemorrhage (ICH). A. 73-year-old woman with CAA-ICH and disseminated cortical superficial siderosis on T2*-GRE (left). Her APOE genotype was ε2/ε4. (B) Multiple strictly lobar cerebral microbleeds (but no siderosis) on T2*-GRE MRI (left) in a 73-year-old woman with cognitive impairment and ε4/ε4 APOE genotype. Note the comparable white matter hyperintensities burden on FLAIR MRI (middle panels) and centrum semiovale perivascular spaces on T2-weighted images (right panel) in the two patients. Both cases had severe CAA with vasculopathic changes.

Among subjects with available genetic testing (n=53), APOE ε2 (but not ε4) allele was overrepresented in cases with disseminated cSS, in the whole cohort (p=0.013), and in the CAA subgroup with ICH (Table 9.3). In logistic regression, disseminated cSS was associated with APOE ε2 (OR: 5.83; 95% CI: 1.49-22.82, p=0.011). These results remained consisted and of similar effect size in a sensitivity analyses adjusting for age and symptomatic ICH clinical presentation (OR: 4.97; 95% CI: 1.11-22.21, p=0.036) and in models further adjusting for the presence of vasculopathic changes on pathology. There was no association between cSS (burden or presence) and APOE ε4. There was no association between CMBs counts and APOE genotype.

Table 9.3 APOE allele prevalences according to cortical superficial siderosis (cSS) burden in cerebral amyloid angiopathy (CAA) patients with or without intracerebral haemorrhage (ICH).

	cSS burden		
	No cSS	**Focal cSS**	**Disseminated cSS**
Whole CAA cohort			
APOE ε2, n (%)	7 (29.2)	3 (25)	12 (70.6)
APOE ε4, n (%)	18 (75)	6 (50)	9 (52.9)
CAA with ICH			
APOE ε2, n (%)	7 (46.7)	1 (11.1)	11 (73.3)
APOE ε4, n (%)	9 (60)	4 (44.4)	8 (53.3)
CAA without ICH			
APOE ε2, n (%)	0 (0)	2 (66.7)	1 (50)
APOE ε4, n (%)	9 (100)	2 (66.7)	1 (50)

Follow-up data were available in all patients presenting without ICH at baseline. During a median follow-up time of 3 years (IQR: 1.1–5.6 years), 2 of 51 patients (3.9%, 95%CI: 0.5-13.5%) experienced a symptomatic lobar ICH. One of these two patients had focal cSS at baseline MRI.

9.4 Discussion

The major findings from this study show that CAA patients presenting with ICH are more likely to have superficial siderosis (particularly disseminated cSS) and the APOE ε2 genotype compared to CAA patients presenting without ICH. By contrast, APOE ε4 is enriched in non-hemorrhagic CAA. In addition, there was an overall higher burden of neurofibrillary tangle pathology in the non-hemorrhagic CAA group. Interestingly, the severity of vascular amyloid pathology did not appear to be different between CAA patients with and without ICH in our cohort, although this could have been influenced by sampling bias. APOE genotype might partly influence these relationships: APOE ε2 was found to be associated with both symptomatic ICH clinical phenotype and disseminated cSS.

Our results provide new insights into the clinical and imaging spectrum of sporadic CAA, as well as potential mechanims and point to different disease phenotypes.[311] While sporadic CAA is commonly found in the elderly,[58] it is currently unknown why only a fraction of people with CAA pathology develop symptomatic disease, and why some present with symptomatic ICH, while others only develop cognitive impairment or other clinical symptoms (but not haemorrhage). A previous comparative post-mortem

histopathological study found that the brain features from CAA patients that are most consistently related to ICH are severe degree of vascular amyloid deposition and the presence of fibrinoid necrosis (with or without microaneurysms).[72] However, cases of CAA with symptomatic ICH in our study had a fairly similar vascular amyloid burden and prevalence of CAA-associated vasculopathic changes compared to cases without ICH. In addition, the two groups had a very similar profile of putative neuroimaging biomarkers of CAA severity, including lobar CMBs presence and number, WMH and high degree of CSO-PVS. It thus seems likely that additional and/or distinct biological pathways from those involved in amyloid-β accumulation in cortical and leptomeningeal vessels play a role in determining clinical expression.

The most distinctive neuroimaging feature between the two groups was the much higher prevalence of disseminated cSS in CAA patients with ICH. The prevalence of cSS in our histopathology-confirmed CAA-ICH group is in line with the reported prevalence in a previous imaging study of CAA-ICH.[301] In addition, the presence of cSS in the group without ICH (10%) is more in line with recent imaging studies evaluating cSS in a memory clinic setting and Alzheimer's disease,[306, 307, 314] and much higher compared to cSS in the population-based Rotterdam Study (0.7% in 1,062 non-demented subjects ≥60 years).[315]

Although the pathophysiological mechanisms underlying cSS in CAA are not yet fully understood, observational data suggest that cSS represents blood residues related to blood leaking episodes into the subarachnoid space from CAA-affected vessels.[190, 195, 301] A 'secondary' mechanism due to leakage or expansion of a lobar ICH into the subarachnoid space cannot be fully excluded; however, similar to other studies, cSS was mostly found distant from any ICH (and often in the opposite hemisphere), and was also detected even in cases without any ICH. In addition, cases in which siderosis was clearly connected to a lobar haematoma were not classified as cSS. Two recent cohort studies have identified cSS as a particular risk factor for subsequent ICH in CAA.[246, 302] In a cohort on 51 patients with CAA-related cSS and a median follow-up of 35.3 months, new intracranial haemorrhages were observed in 24 patients (47.1%).[246] The majority of new haemorrhages (i.e. 13/18 new ICH and 4/6 new acute convexity subarachnoid bleeds) were located immediately adjacent to pre-existing cSS.[246] A European multicentre cohort of probable or possible CAA patients (n=118; median follow-up 24 months), found that cSS was a significant predictor of time until ICH.[302] The ICH risk at 4 years was 25% (95%CI: 7.6-28.3%) for patients without siderosis, 28.9% (95%CI: 7.7-76.7%) for patients with focal siderosis and 74% (95%CI: 44.1-95.7) for patients with disseminated cSS (log-rank test: p=0.0031).[302] A small autopsy series of six CAA cases showed that multiple leptomeningeal arteries (and not parenchymal cortical vessels) can rupture into the subarachnoid space and the brain parenchyma, leading

to large lobar haemorrhages.[248, 316] All patients had multiple haematomas in the subarachnoid space (mainly in cerebral sulci), as well as intracerebral haematomas, and each intracerebral haematoma was connected to the subarachnoid haematomas at the depth of cerebral sulci or through the lateral margin of the cortex.[248] In this neuropathological study there was evidence of only few ruptured cortical arteries associated with lobar ICH. These observations might explain the association between disseminated cSS and CAA patients with ICH in the present study, as well as why patients with disseminated cSS have the highest risk of future ICH.[302]

In addition, our study demonstrates that there might be APOE genotype-specific effects on the imaging and clinical expression of CAA-related disease. While APOE ε4 seems to be more associated with CAA without ICH, APOE ε2 is linked more strongly with bleeding and cSS. The APOE ε2 allele was previously reported to be associated with CAA-related lobar ICH, possibly owing to the disease-related vasculopathic abnormalities seen with this allele,[83, 88] and recently with cSS in a cohort of CAA defined by clinico-radiographic criteria.[317] APOE ε4 is also associated with risk of sporadic CAA and ICH, the severity of which depends on the number of APOE ε4 alleles.[77, 318, 319] APOE ε4 might increase CAA severity by enhancing amyloid-β deposition within small cerebral vessels whilst APOE ε2 promotes the vasculopathic changes which can lead to vessel rupture.[80, 88] Our study raised the interesting possibility APOE ε2 influences pathways causing both cSS and ICH. Interestingly, after accounting for the presence of vasculopathic changes, the apparent relation of APOE ε2 to cSS was not reduced, suggesting that vascular amyloid load and how extensively the vessel wall architecture is disrupted is not the sole driving force underlying these pathways and hence, these clinical phenotypes. However, any mechanistic links are clearly complex and need to be treated with caution, as APOE genotype alone might not be necessary or sufficient to cause or drive these effects. It is important to note that links may differ according to the presence or absence of Alzheimer's disease pathology, particularly for APOE ε2, which has been associated with a reduced risk of late-onset Alzheimer's dementia.[77] This observation is in line with the differential associations between neuritic plaques and neurofibrillary tangles data in our CAA patients with and without ICH. Indeed, there is significant variation in the pathological appearance of CAA and its influence on Alzheimer's disease pathology,[320, 321] further highlighting the concept of different phenotypes of CAA which can follow and influence several different pathways. It is important to acknowledge thought that the overrepresentation of neurofibrillary tangles among the non-ICH CAA patients might also partly reflect a bias issue, since by definition these cases presented cognitive impairment, partly driven by neurofibrillary tangle pathology.

Although the natural history of symptomatic CAA patients mainly presenting with cognitive impairment (i.e. without ICH at baseline) has not been fully investigated, our data suggest that this CAA phenotype may have a lower risk of developing ICH. It is important to note that CAA patients without major ICH (microbleeds- or cSS-only CAA) presenting to stroke services with symptoms other that cognitive impairment (e.g. transient focal neurological episodes), might still be at significant risk of future bleeding.[244, 302] A recent study on patients presenting with isolated lobar microbleeds showed that they indeed have a hemorrhagic risk profile suggestive of severe CAA pathology.[322] However, the ICH risk among CMB-only CAA patients was lower compared to CAA patients with past ICH. This topic requires further investigation in prospective studies, especially given the increased incidence of ICH in patients with Alzheimer's disease and the biological association of Alzheimer's disease with CAA.[323, 324] Whether patients with CAA-related cognitive impairment/Alzheimer's disease and cSS have a distinct clinical sub-phenotype (e.g. worse outcome, high APOE e2 genotype frequency, increased ICH) also requires further study.

The main strengths of this study include the large sample of patients with histopathologically confirmed CAA and available MRI sequences, the systematic evaluation of MRI scans for a range of imaging markers of small vessel disease, and the testing of a prespecified hypothesis. The main limitations, inherent to any clinical-pathology series in CAA, include: (a) the difference in pathology sampling between autopsied brains and biopsies/haematomas; (b) the biases regarding which patients get biopsied or autopsied which might be skewed towards end stage or advanced disease; and (d) potential selection bias due to the requirement for both pathology and MRI and the unavailability of APOE genotype data in all patients. The differences in Alzheimer's pathology between groups could potentially be explained by a longer duration of disease (taking time from MRI to pathology as a surrogate for disease duration) in CAA patients without ICH or the different characteristic between the two groups (including APOE ε4 prevalence). Further molecular imaging studies assessing amyloid and tau burden *in vivo*[325] may help resolve this question. Also, the cross-sectional design of the current study did not allow us to assess potential causality of the reported associations.

In summary, results from this neuropathological-defined cohort of CAA patients with and without ICH provide multiple lines of evidence for distinct disease phenotypes, and suggest that cSS and APOE ε2 are related to the haemorrhagic expression of the disease. Our study emphasizes the widening spectrum of CAA with clinical phenotypes reflecting different neuroimaging and genetic features and suggests divergent pathophysiological mechanisms. Although these findings require external validation in larger CAA cohorts, they may be relevant for future biomarkers studies and disease-modifying CAA trials.[300]

Chapter 10 Cerebral microbleeds, microangiopathy subtype and recurrent spontaneous intracerebral haemorrhage risk: systematic review and meta-analysis

ABSTRACT

Objective: To evaluate cerebral microbleeds (CMBs) and recurrent intracerebral haemorrhage (ICH) and ischaemic stroke risk in spontaneous ICH survivors, stratified by the MRI-defined presumed underlying microangiopathy – i.e. cerebral amyloid angiopathy (CAA) vs. non-CAA related ICH.

Methods: Meta-analysis of prospective cohorts with recent spontaneous ICH. We estimated annualised recurrent symptomatic ICH and ischaemic stroke rates for each study and pooled odds ratios of recurrent ICH and ischaemic stroke across CMBs groups (i.e. 1, 2-4, 5-10 and >10 CMBs), using a random effects model.

Results: We pooled data from 8 hospital-based studies including 981 patients: 3 CAA-related ICH cohorts (n=248) and 6 non-CAA related ICH cohorts (n=733). The annual risk of recurrent ICH was higher in patients with CAA-related ICH compared with non-CAA related ICH cohorts (9.96%; 95%CI: 5.08-14.84 *vs.* 1.31%; 95%CI: 0.51-2.11 respectively, p=0.012). In non-CAA related ICH cohorts, only patients with >10 CMBs had an increased risk of recurrent ICH (OR: 5.63; 95%CI: 1.79-17.72; p=0.003); CMBs located in both deep and lobar regions the stronger association with recurrent ICH (OR: 4.01; 95%CI: 1.47-10.94; p=0.007). In CAA-related ICH cohorts higher numbers of baseline CMBs strongly predicted an increased risk of recurrence during follow-up: the cumulative OR was 3.32 (95%CI: 1.41-7.81; p=0.006), 4.43 (95%CI: 1.76-11.14; p=0.002) and 3.03 (95%CI: 1.19-7.74; p=0.02) for 2-4, 5-10 and >10 CMBs respectively. This risk of recurrent ICH was not significant for patients with a single CMB in any of the cohorts. CMBs were not associated with ischaemic stroke risk in either CAA or non-CAA related ICH cohorts. The relative risk for recurrent ICH vs. ischaemic stroke in non-CAA ICH patients with CMBs did not differ significantly. By contrast, in CAA-related ICH patients with CMBs, the relative risk of recurrent ICH compared to ischaemic stroke was 31.2 (95% CI: 5.9-164.6; p<0.0005).

Conclusions: In ICH patients, multiple CMBs at baseline are associated with an increased ICH recurrence risk. The recurrent ICH risk in those with MRI-defined CAA-related ICH is about 8 times higher than for non-CAA related ICH. The balance of risk for recurrent ICH vs. ischaemic stroke also differs between CAA and non-CAA related ICH cohorts. Defining ICH subtypes using MRI is relevant for prognosis, and should be considered in designing future ICH clinical prevention trials.

10.1 Introduction

Spontaneous intracerebral haemorrhage (ICH) presumed to be due to cerebral small vessel disease[31] is one of the most catastrophic forms of stroke.[326] It is associated with high morbidity and mortality,[327] but also a substantial risk of recurrence for survivors.[220, 253, 254] Understanding the causes and pathophysiology of spontaneous ICH can help stratify the risk of recurrent ICH or ischaemic cerebrovascular events with relevance for the development of new treatments. Two main disease processes can cause rupture of small arteries of the brain leading to spontaneous ICH: hypertensive arteriopathy and sporadic cerebral amyloid angiopathy (CAA).[2] Hypertensive arteriopathy is characterized by lipohyalinosis and fibrinoid necrosis of small deep perforating arteries, resulting in haemorrhages particularly in deep brain regions, such as the basal ganglia and thalamus.[2] CAA affects the small cortical and leptomeningeal arteries, by the progressive deposition of amyloid-β in the vessel walls and is an important cause of lobar (but not deep) ICH, especially in the elderly.[28, 328] These two common small vessel pathologies that result in ICH seem to have intrinsically different risks of recurrent haemorrhage: a history of CAA-related lobar ICH might carry a significantly higher risk for recurrence compared to deep ICH due to hypertensive arteriopathy,[254, 255, 329] but these risks are currently difficult to predict.

With the development of more advanced imaging techniques over the past decades, new neuroimaging markers of small vessel disease have been emerged. Among them, cerebral microbleeds (CMBs) on blood-sensitive MRI sequences, including T2*-weighted gradient-recalled echo (T2*-GRE) and susceptibility-weighted imaging (SWI), have become a relatively common finding in patients with spontaneous ICH and warfarin-related ICH.[35, 212] By contrast with other markers of small vessel disease (e.g. leukoaraiosis or lacunes), CMBs may provide direct evidence of blood leakage from haemorrhagic-prone microangiopathy.[172, 330] The presence of CMBs is also regionally associated with ICH, making them a possible marker of more bleeding-prone areas.[177] In addition, the location of CMB in the brain appears to reflect the type of the underlying microangiopathy: similar to macrobleeds, strictly lobar CMBs (cortical-subcortical) are characteristic of CAA-related small vessel disease (allowing the diagnosis of CAA during life using the Boston criteria[202, 203]), whilst deep CMBs appear to reflect hypertensive arteriopathy.[27, 35] If the risk of ICH recurrence is related to the underlying arteriopathy type and burden, CMBs may be of particular value in identifying patients at high risk of recurrence.

Some studies indeed show that the burden of CMBs predicts the risk of recurrent ICH; the relationship with the risk is stronger in patients with CAA-related lobar ICH.[180, 227, 331] However, these studies are too small to guide patient management at present; and more reliable estimates of the risks are urgently needed. We therefore performed a meta-analysis of all available evidence from published prospective ICH cohorts investigated using MRI, to investigate the power of CMBs (including CMBs number and location) to predict recurrent ICH, stratified by the presumed underlying microangiopathy (CAA vs. non-CAA related ICH). Since CMBs could also be associated with occlusive features of cerebrovascular disease, as a secondary analysis we also explore their relation with incident ischaemic stroke in this patient population.

10.2 Methods

10.2.1 Search strategy, selection criteria and data extraction

PubMed was searched between January 1, 1999 and November 1, 2013 using medical subject heading (MESH) terms and text words: ["microbleed*" OR "microh(a)emorrhage*" OR "h(a)emorrhagic lacune" AND ["stroke" OR "intracerebral h(a)emorrhage" OR "brain h(a)emorrhage"]. Reference lists from all included articles, relevant review articles and the authors' own files were also searched for additional eligible reports. Studies were eligible for inclusion if they: included adult patients with spontaneous symptomatic ICH confirmed by imaging; had a prospective design, with at least three months of follow-up; assessed the risk of recurrent symptomatic spontaneous ICH (main outcome) during follow-up; had data for the presence of CMBs on baseline T2*-GRE MRI; and were published in English. In cases of multiple publications from the same or overlapping cohorts, only the most recent comprehensive results from the report with the largest sample size were generally used in the analysis. We excluded case-control and cross-sectional studies, case reports or case-series, population-based studies and studies in other patient populations (e.g. CADASIL, thrombolysis-related ICH etc.).

For each study, we extracted data on the country of the study, time period, clinical setting, population size, demographic data (including mean age and sex and vascular risk factors), use of antithrombotic agents, T2*-GRE MRI parameters, number of participants with at least one CMB at baseline, method and duration of follow-up and number of participants with the outcome events of interest (recurrent spontaneous symptomatic ICH

and symptomatic ischaemic stroke, clearly defined according to defined criteria). For included cohorts we sought further information from the authors (by email) on total person-years of follow-up and outcome events (recurrent ICH and symptomatic ischaemic stroke) stratified by CMBs numbers (1 CMB, 2-4, 5-10 and >10 CMBs) and location (lobar - in the cortex or subcortical areas of the cerebral hemispheres , deep or mixed CMBs). We classified each study cohorts as CAA-related ICH or non-CAA related ICH according to classic Boston criteria,[202] based on published information of included subjects in each study and correspondence with authors.

10.2.2 Statistical analysis

We estimated annualised recurrent symptomatic ICH and ischaemic stroke rates (%/year) and corresponding 95% confidence intervals (CI) for each study from a poisson regression model and exact poisson intervals. Pooled rates were calculated using an inverse variance-based random effects method (DerSimonian-Laird) and stratified by study population (presumed underlying small vessel disease cause of baseline ICH, i.e. CAA-related ICH vs. non-CAA ICH). The log(incidence) of recurrent ICH events where compared between these groups using a significance test with the appropriate degrees of freedom.

We meta-analysed data for recurrent ICH and ischaemic stroke across studies, using a random effects model with DerSimonian-Laird weights,[332] quantifying the strength of any association using odds ratios (OR) and its associated 95% CI in patients without CMBs vs. different CMBs groups. To maximise the power of our analyses, for comparisons with zero events in both groups, we added 0.5 to each group, considered OR=1 and calculated calculating the standard error (SE), logOR and SE logOR by using the 2-variable input method. We assessed heterogeneity by I-squared and x-squared statistics and also visually through inspection of the forest plot and checking for overlapping confidence intervals. We explored publication bias with funnel plots and the Harbord regression tests for funnel plot asymmetry. Analyses were stratified by baseline ICH presumed cause (CAA vs. non-CAA related ICH). As a sensitivity analysis we investigated the influence of one study on the overall meta-analysis estimate (using the "metaninf" command) and inspect the results graphically with meta-analysis estimates computed, omitting one study in each turn. We used meta-regression to explore whether certain confounders could have affected our results.

All meta-analyses were performed using Stata 11.2 (StataCorp LP, Texas). We prepared this report with reference to the Preferred Reporting Items for Systematic

reviews and Meta-Analyses (PRISMA)[333] and the Meta-analysis of Observational Studies in Epidemiology (MOOSE)[334] guidelines.

10.3 Results

Eight unique hospital-based studies with a total of 981 patients (665 with CMBs) met our predefined inclusion criteria,[227, 302, 331, 335-339] and were pooled in meta-analyses. The studies comprised of three CAA-related ICH cohorts (n=248) [227, 302, 337] and six non-CAA related ICH cohorts (n=733)[331, 335, 336, 338, 339] (i.e. one study included both cohorts[227]). The basic characteristics of included cohorts are summarised in Table 10.1. Most studies had similar inception points (i.e. the start of follow-up), methods of assessment and a high number of participants lost to follow-up. Most studies used reliable ascertainment methods for establishing recurrent ICH events at the follow-up.

In these studies, the overall pooled annual risk of recurrent ICH and ischaemic stroke was 3.20% (95% CI: 1.52-4.88) and 0.87% (95% CI: 0.25-1.48) respectively (Figure 10.1). Patients with CAA-related ICH had a higher annual risk of recurrent ICH compared with non-CAA related ICH cohorts (9.96%; 95% CI: 5.08-14.84 vs. 1.31%; 95% CI: 0.51-2.11 per year respectively, p=0.012). The ischaemic stroke rates could not be directly compared in the two patient groups, because in one CAA cohort ischaemic stroke was not an outcome event,[337] and in another study no ischaemic strokes were observed.[227] However, individual non-CAA ICH cohorts seem to have a higher risk of ischaemic stroke at follow-up when indirectly compared with the CAA cohorts.

All studies used T2*-GRE MRI to detect CMBs at baseline, although imaging parameters, including echo time and slice thickness varied slightly (Table 10.1). Differences in demographic, clinical and imaging characteristics between the CMB (+) vs. CMB (-) groups are noted in Table 10.1. Among patients with CMBs, 79/665 (11.9%; 95% CI: 9.5-14.6%) experienced recurrent ICH during follow-up, compared to 16/316 (5.1%; 95%CI: 2.9-8.1%) patients without CMBs. The results of the overall analysis showed that those with any baseline CMBs had a higher risk of recurrent ICH at follow-up (OR: 2.81; 95% CI: 1.61-4.93); p<0.0005) (Appendix F Figure 1). This risk was not significant for patients with a single CMB compared to those without CMBs. However, amongst different multiple CMBs groups (i.e. 2-4, 5-10 and >10 CMBs), there was a graded relationship between CMBs burden and higher recurrent ICH risk (Appendix F Figure 1). The results were consistent from study to study (test for heterogeneity p>0.10).

Table 10.1 Basic study design and patients characteristics of included studies.

Study	Country/ Setting	T2*-GRE MRI parameters (Field strength/ET/ST)	Patient number (% men)	Inception point	Mean age (SD)	HTN	Hx of ICH	Antithrombotic users		Advanced WMC (grade 2 or 3)	CMBs prevalence	Significant differences CMBs(+) vs. CMBs(-) groups
								Antiplatelets	Oral anticoagulants			
Non-CAA related ICH cohorts												
Kang *et al* 2012[335]	South Korea Multicentre	1.5T/30, 20,23ms/1-2mm	97 (60.8%)	ICH onset (within 3 d)	59.1y (12.1)	84.5%	5.2%	14.4%	0%	42.2%	76.3%	Age: 62(26-81) vs. 53(38-77); p=0.059 Advanced WMH: 52.7% vs. 8.7%; p<0.001
Imaizumi *et al* 2012[336]	Japan Single centre	1.5T/26ms/7.5mm	187 (57.8%)	ICH onset (within 7 d)	68.4y (11.9)	67.4%	9.1%	5.9%	3.2%	28.9%	71.1%	No
Biffi *et al* 2010[227]	USA Single centre	1.5T/50ms/5-6mm	104 (58.5%)	90 d after onset	70.2y (12)	81.7%	2.9%	40.4%	19.2%	75%	58.7%	No
Jeon *et al* 2007[331]	South Korea Single centre	1.5T/30ms/2mm	63 (66.7%)	ICH onset (within 10 d)	58.3y (range 38-81)	96.8%	NA	6.4%	0%	NA	68.3%	Hypocholesterolemia 37.2% vs. 5%; p= 0.013
Naka *et al* 2006[338]	Japan Single centre	1T/26ms/5mm	83 (65.1%)	Hospital discharge	63.5y (11.7)	83.1%	18.1%	2.4%	2.4.%	28.9%	48.2%	Advanced WMH: 45% vs. 14%; p=0.002
Imaizumi *et al* 2004[339]	Japan Single centre	1.5T/26ms/8mm	199 (50.8%)	Days after onset	65.9y (10.9)	84.4%	9.1%	1%	0%	NA	77.4%	Age: 67.3±10.8 vs. 61.4±11.2; p=0.0013
CAA-related ICH cohorts												
Charidimou *et al* 2013[302]	UK/Belgium Multicentre	1.5T/15-70ms/5mm	104 (48.1%)	MRI (median 17 d post ICH)	71.3y (9.5)	59%	28.9%	20%	2%	44.1%	65.4%	No
Domingues-Montanari *et al* 2011[337]	Spain Single centre	1.5T/29ms/?mm	40 (45%)	1 month after onset	74.7y (7)	43.6%	45%	NA	NA	51.3%	70%	Previous ICH: 53.6% vs. 16.7%; p=0.041
Biffi *et al* 2010[227]	USA Single centre	1.5T/50ms/5-6mm	104 (58.7%)	90 d after onset	72.5y (8.2)	58.2%	7.7%	15.4%	10.6%	71.2%	60.6%	HTN: 63.4% vs. 36.6 %; p=0.007

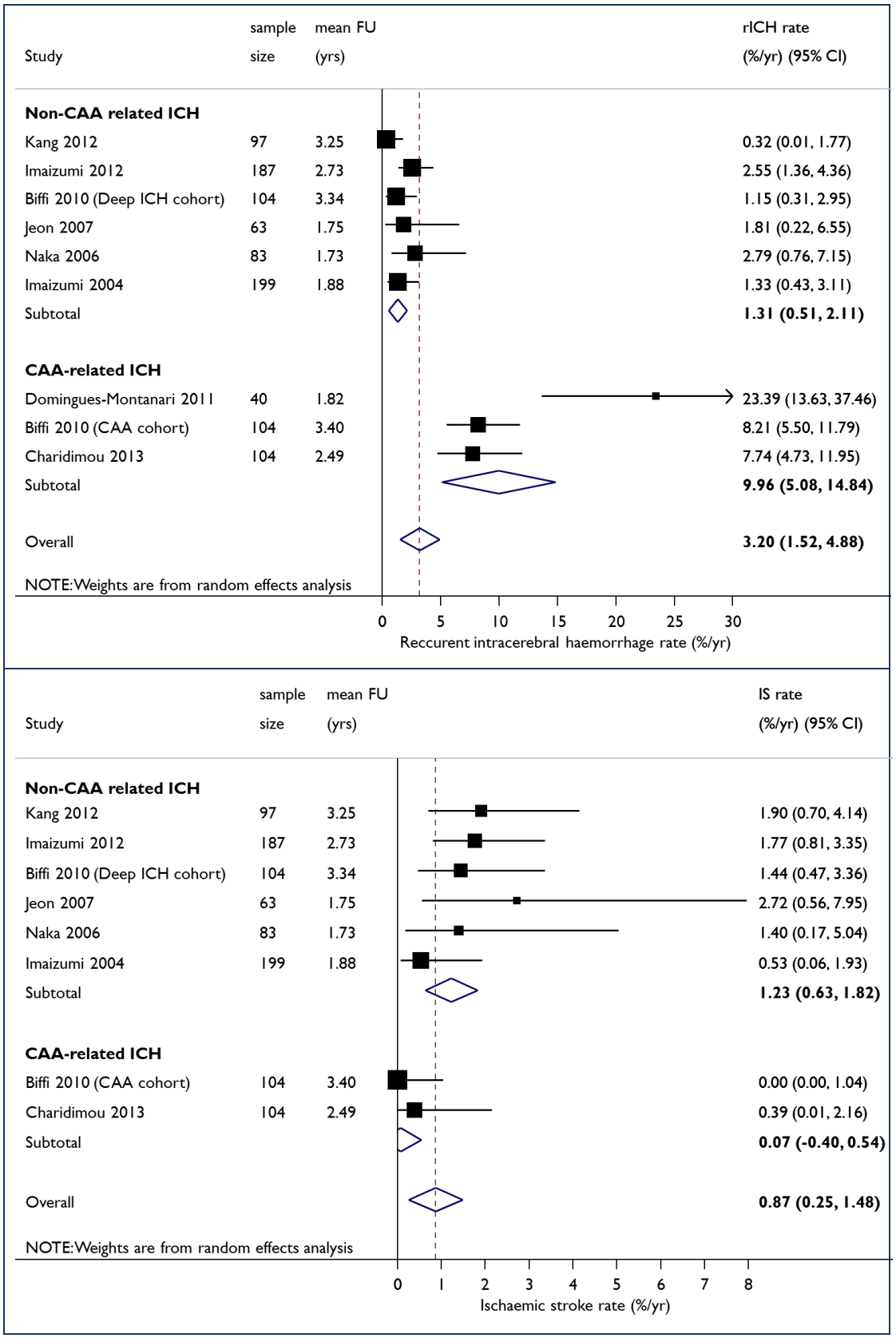

Figure 10.1 Pooled risks of recurrent symptomatic intracerebral haemorrhage (top panel) and ischaemic stroke (lower panel) during follow-up in included studies.

In the non-CAA related ICH cohorts, amongst patients with CMBs, 26/506 (5.1%; 95% CI: 3.4-7.4%) experienced recurrent ICH, compared to 3/227 (0.8%; 95% CI: 0.3-3.8%) patients without CMBs. The presence of CMBs was marginally related to an increased risk of recurrent ICH risk (OR: 2.63; 95% CI: 0.99-6.99; p=0.053) (Figure 10.2). Although there was a trend for higher risk across different CMBs burden groups compared to no CMBs, this risk was more pronounced only for patients with >10 CMBs (OR: 5.63; 95% CI: 1.79-17.72; p=0.003) (Figure 10.2). When we meta-analysed data based on CMBs location in the brain (strictly lobar, strictly deep, mixed CMBs etc.), the presence of any lobar and mixed CMBs (but not strictly lobar or strictly deep) were associated with a risk of recurrent ICH (OR: 3.66; 95% CI: 1.31-10.27; p=0.014 and OR: 4.01; 95% CI: 1.47-10.94; p=0.007 respectively) (Figure 10.3). The presence of any deep CMBs were marginally associated with a high risk of ICH recurrence (Figure 10.3).

In the CAA-related ICH cohorts, 53/159 (33.3%; 95% CI: 26.1-41.2%) patients with CMBs and 13/89 (14.6%; 95% CI: 8-23.7%) patients without CMBs suffered recurrent lobar ICH during follow-up. The pooled OR for CMBs presence and recurrent ICH risk was 2.91 (95% CI: 1.47-5.77; p=0.002) (Figure 10.4). The presence of a single CMB was not associated with a higher risk of recurrence compared to CAA patients without any CMBs (OR: 1.71; 95% CI: 0.63-4.66; p=0.294). However, the cumulative OR for recurrent ICH during follow-up was 3.32 (95% CI: 1.41-7.81; p=0.006) for subjects with 2-4 CMBs, 4.43 (95% CI: 1.76-11.14; p=0.002) for 5-10 CMBs and 3.03 (95% CI: 1.19-7.74; p=0.02) for more than 10 CMBs (Figure 10.4). Further analyses within these groups revealed no differences in the risk for each increase in category and for 2-4 CMBs vs. ≥5 CMBs groups (RR: 1.035; 95% CI: 0.66-1.62; p=0.879) (data not shown).

In a secondary analysis, CMBs (including presence and different number groups) were not associated with ischaemic stroke risk in either CAA or non-CAA related ICH cohorts. In a direct comparison, the relative risk for recurrent ICH vs. ischaemic stroke in patient with CMBs, was not significant for non-CAA related ICH cohorts across different CMBs groups. In patients with CAA-related ICH and any CMBs, the relative risk of recurrent ICH compared to ischaemic stroke was 31.2 (95% CI: 5.9-164.6; p<0.0005). Even though no heterogeneity was detected overall we used meta-regression to see whether certain confounders could have affected our results. No significant heterogeneity was noted according to age, gender, hypertension at baseline, history of previous ICH or white matter changes for the main outcomes. Sensitivity analyses involving sequential removal of each individual study in turn yielded very similar results for all comparisons. Estimation of publication bias via the Egger test and the Begg test returned non-significant results (all p>0.20).

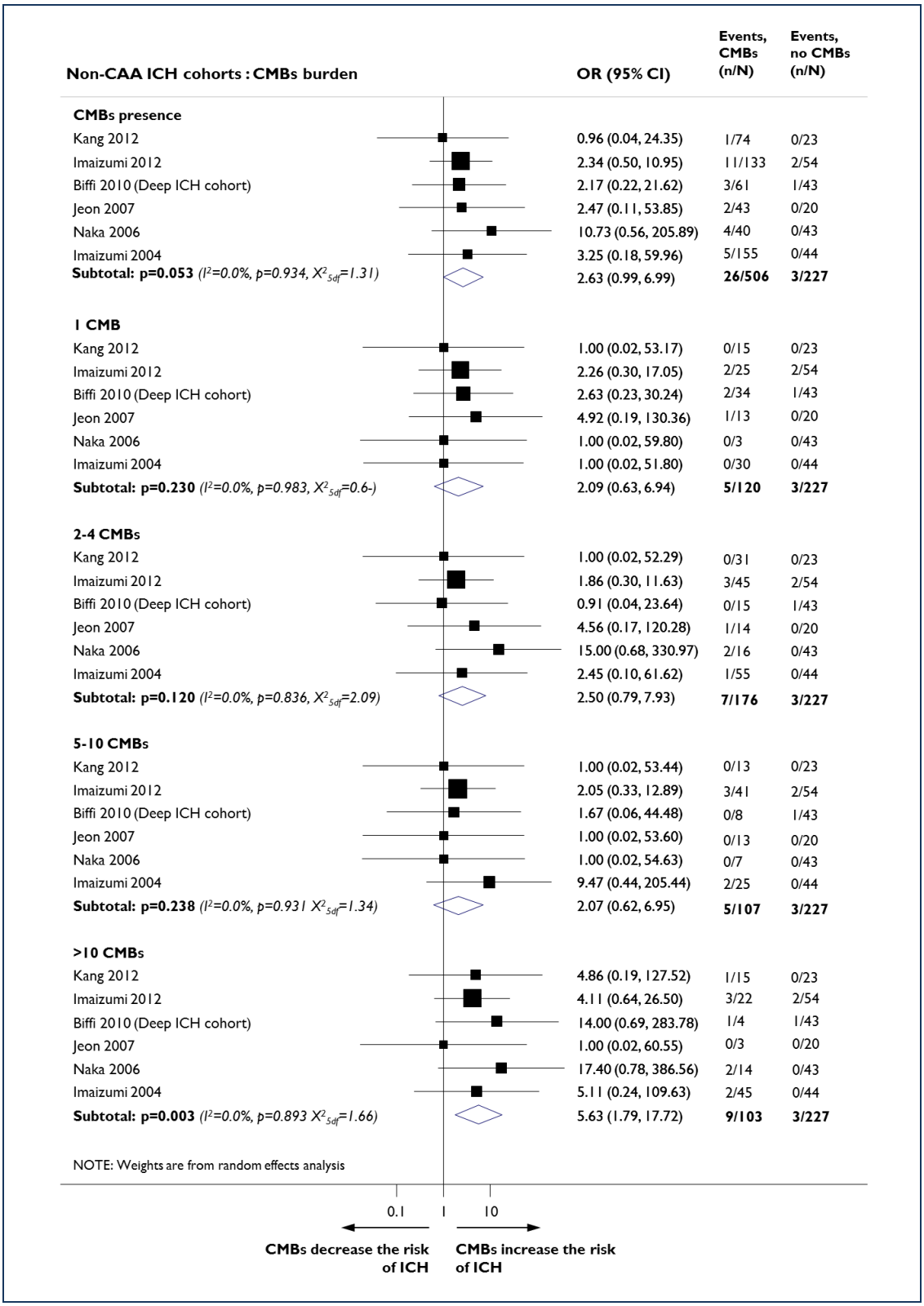

Figure 10.2 Meta-analysis of effect of cerebral microbleeds (CMBs) presence and burden on the risk of recurrent spontaneous ICH in non-CAA ICH cohorts.

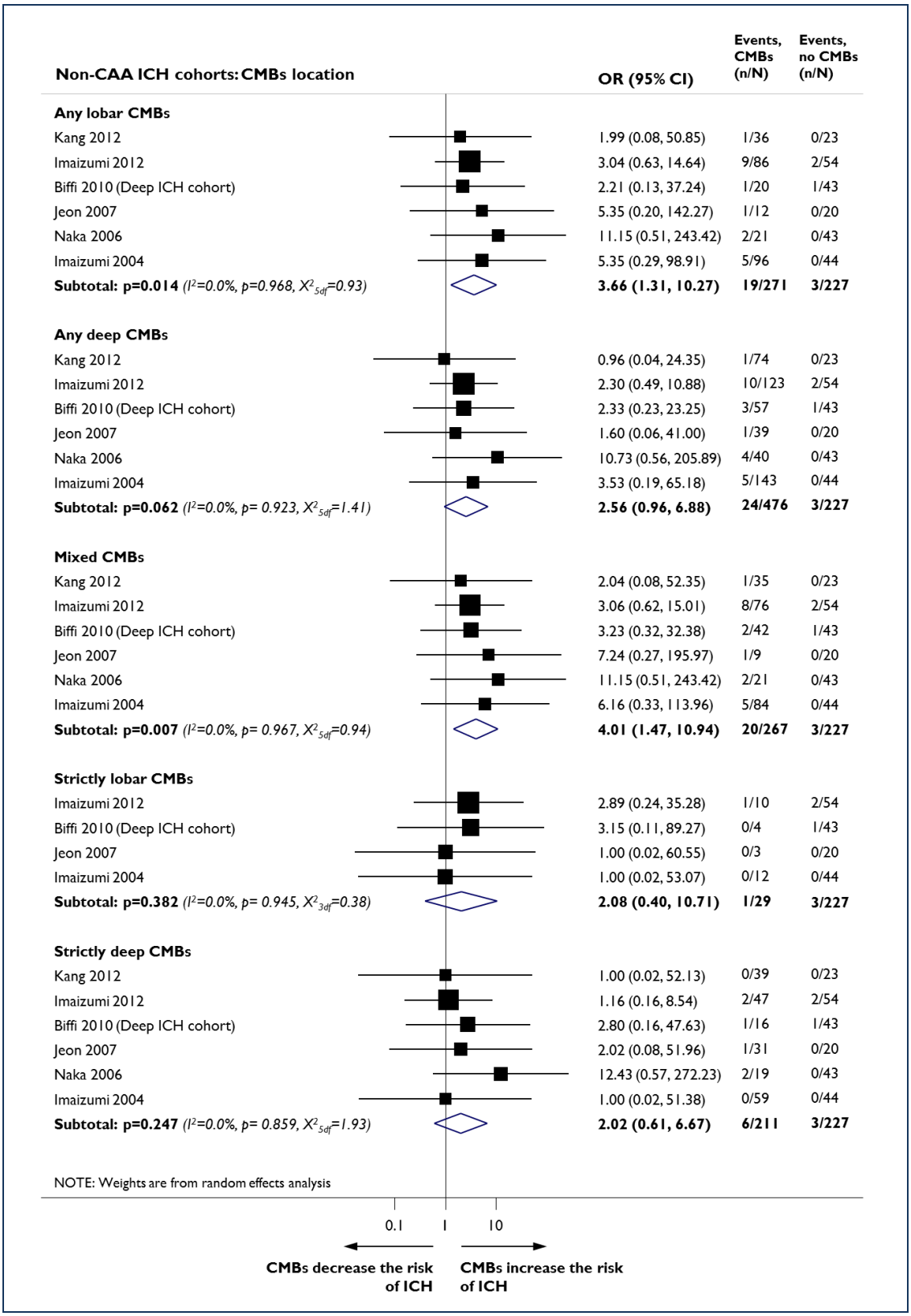

Figure 10.3 Meta-analysis of effect of cerebral microbleeds (CMBs) location on the risk of recurrent spontaneous ICH in non-CAA ICH cohorts.

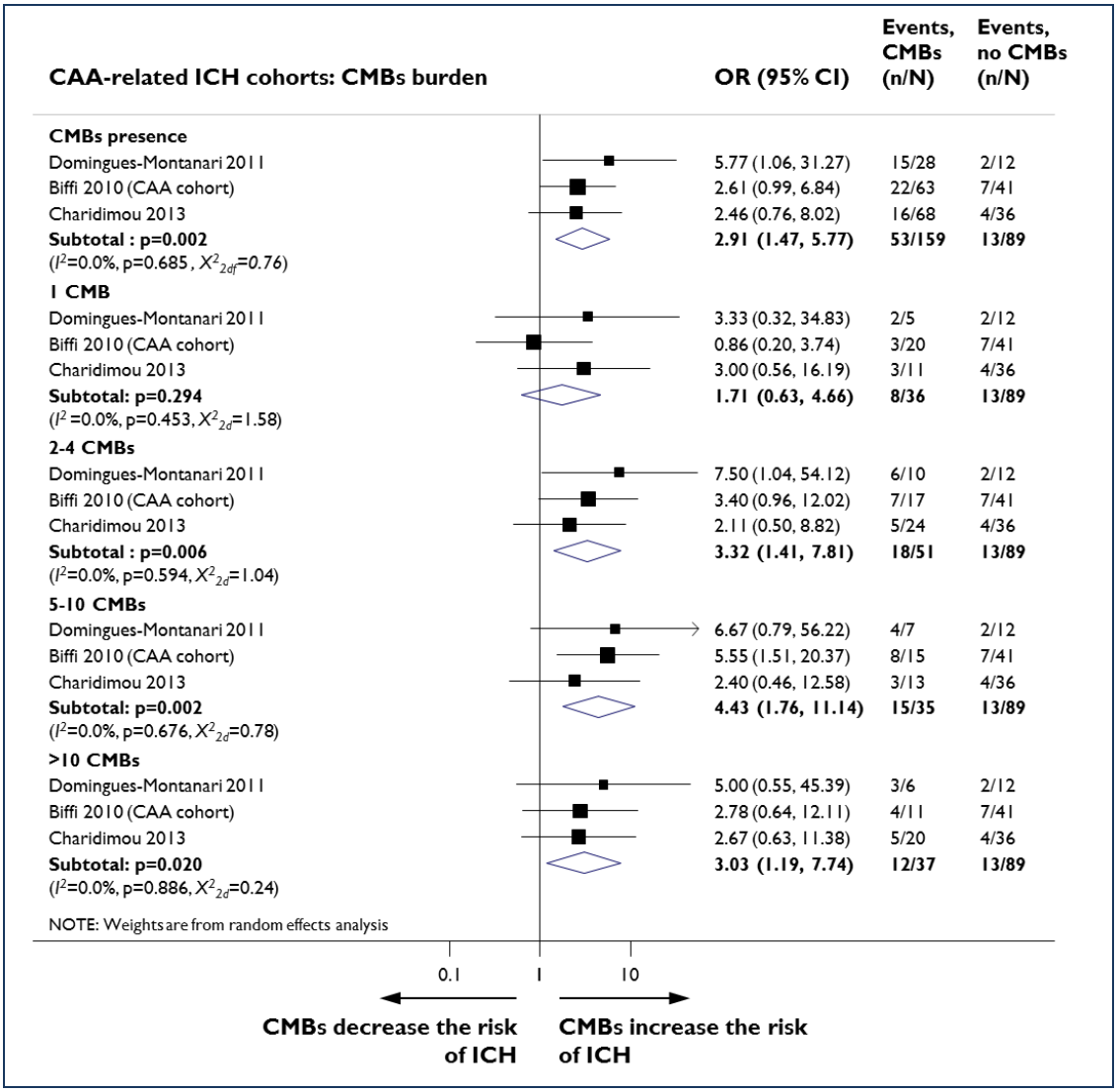

Figure 10.4 Meta-analysis of effect of cerebral microbleeds (CMBs) presence and burden on the risk of recurrent spontaneous ICH in CAA ICH cohorts.

10.4 Discussion

In this systematic review and meta-analysis of eight cohorts involving 981 survivors with symptomatic spontaneous ICH, the pooled estimates demonstrated a consistent association between CMBs presence at baseline and future ICH recurrence. However, the strength of the association and the magnitude of recurrent ICH risk, especially in relation to CMBs burden and location, were considerably different depending on the nature and severity of the underlying microangiopathy – i.e. CAA-related and non-CAA related (presumably hypertension-related).

Based on data from included cohorts the aggregate rates of recurrent ICH was about 8-fold higher in patients with presumed CAA-related lobar ICH, compared to non-CAA

ICH. This is in line with most previous studies[149, 340, 341] and a systematic review[254] that have found ICH recurrence is more common following lobar ICH than non-lobar ICH (i.e. deep ICH) (4.4% vs. 2.1% per year respectively; p=0.002). In the cited review, the risk of ischaemic stroke (1.1% per year) was lower than the overall risk of recurrent ICH.[254] A more recent meta-analysis also confirmed that ICH survivors seem to be at considerable risk of serious vascular events, with recurrent ICH being more frequent after lobar ICH.[327] In contrast with the previous systematic review, ischaemic stroke appeared to be at least as common as recurrent ICH over 3 years in four studies with appropriate data.[327]

However, the included studies in these meta-analyses[254, 327] did not have the benefit of MRI to systematically phenotype the likely underlying arteriopathy. The causal small vessel disease might have implications for prognosis and treatment, for example, the potential benefits and hazards of antithrombotic drugs (including antiplatelets and oral anticoagulant).[342, 343] In our meta-analysis, the risk of ischaemic stroke was negligible for CAA patients; but nearly as high as the risk of recurrent ICH in the non-CAA cohorts. The prominent rate of recurrent CAA-related ICH and the different rates of subsequent ischaemic stroke compared to non-CAA related ICH, suggest that vascular mechanisms (and relative risk of bleeding vs. ischaemia) are fundamentally different between these two subtypes of small vessel disease.[2] It is important to remember that hypertensive small vessel disease resulting in haemorrhages in deep brain areas, and hypertension in general (which was more prevalent in non-CAA compared to CAA cohorts; see Table 1), is also a major risk factor for ischaemic cerebrovascular events. By contrast, CAA is not accounted by conventional vascular risk factors (such as hypertension) and seems to be a more active haemorrhage-prone microangiopathy compared to hypertensive arteriopathy: the progressive accumulation of vascular amyloid-β causes weakening and cracking of cortical and leptomeningeal vessel walls, resulting in haemorrhage.[28] History of previous ICH was more common in CAA compared to non-CAA cohorts in our meta-analysis. While CAA is associated with presumed asymptomatic cerebral microinfarcts, symptomatic ischaemic strokes are rare.[197, 328] These characteristics, as well as variation in the use of antihypertensive therapy might partly account for the different stroke risks in the two groups.

The differential stroke risks in ICH survivors have implications for prognosis and secondary prevention decision-making, especially regarding whether to prescribe antithrombotic treatments in individuals with a history of ICH, an increasingly common clinical dilemma.[342, 344] The key question is whether CMBs are linked to an increased risk of "macrobleeding", but available data for this hypothesis are limited.[345] The high prevalence of CMBs in patients with ICH and the relatively low recurrence rates of ICH we found,

suggest that there might be only specific sub-group of patients with CMBs that are at particularly high risk for further haemorrhagic events. Our meta-analysis of ICH cohorts provides new and stronger evidence for this assumption.

Perhaps unsurprisingly, we did not find a significant association between the presence of only one CMB and future recurrent ICH risk in the whole ICH population and also in neither CAA-related, nor non-CAA-related ICH cohorts. However, the pathophysiological significance of a single CMB is unclear: it might indicate a less severe or less active microangiopathy, or it might be just the tip of the iceberg of small vessel damage. This issue is further complicated because the classification of patients into those having single *vs.* multiple microbleeds may vary with MRI characteristics used: e.g. higher field strengths, thinner slices, or susceptibility-weighted imaging seem to increase sensitivity to CMBs detection.[346] All of the included studies have relied on "conventional" T2*-GRE MRI performed under standard imaging parameters, commonly used in clinical practice. Also, in one microbleed rating scale validation study, patients with only one potential CMB accounted for most cases of disagreement and inter-rater variability.[238]

We have confirmed that CAA-related lobar CMBs are a strong predictor of recurrent ICH on clinical follow-up, making them good prognostic biomarkers in CAA. Despite this overall association, the occurrence of CAA-related recurrent lobar ICH was considerably lower than the overall prevalence of lobar CMBs at baseline. This observation indicates that particular properties of the CAA-affected vessels as well as additional factors may determine whether a vessel rupture will result in a CMB or a larger macrobleed.[198] However, underscoring a connection between microbleeding and macrobleeding, the dynamic development of new CMBs on serial T2*-GRE MRI over time also predicts subsequent recurrent ICH in CAA.[180] Recent studies have shown most of these new CAA-related haemorrhages arise at sites of high baseline amyloid-β deposition, detected *in vivo* using PiB PET imaging.[176, 285] It is thus possible that in certain circumstances (e.g. with the use of antithrombotic drugs) CAA-related CMBs, rather than being sealed off by normal hemostatic mechanisms and surrounding tissue, may enlarge into a symptomatic lobar ICH, underlying the strong association between CAA and anticoagulant-related ICH.[152, 347, 348] Within this context, a decision analysis based on a 69-year-old survivor of a lobar CAA-related ICH and newly diagnosed non-valvular atrial fibrillation suggested that should not be anticoagulated with warfarin across the spectrum of thromboembolic and haemorrhagic risks.[349] The strong association of lobar CMBs burden and CAA-related recurrent ICH we demonstrated, as well as the association with anticoagulation-related ICH[152] makes them an important feature for haemorrhagic risk assessment in older patients at high risk of CAA pathology. However, it remains unclear whether the presence of strictly lobar CMBs in the

absence of symptomatic lobar ICH (i.e. microbleeds-only CAA) which are highly prevalent in the elderly,[174] confers sufficient risk of future ICH. A recent study indicates that this incident ICH risk is not trivial and warfarin might be a significant predictor of ICH occurrence in this population ($p<0.05$) but definitive data are lacking.[350]

In the non-CAA related ICH cohorts, our results indicate that a certain CMBs load, specifically the presence of more than ten CMBs, and a mixed distribution in the brain, are a strong marker of recurrent ICH risk. Since hypertensive small vessel damage primarily affects deep brain regions, the presence of both deep and lobar CMBs is an indication of advanced and widespread disease. In all the other CMBs groups the risk of recurrent ICH might be at least as important as the risk for future ischaemic stroke (and probably all vaso-occlusive events).[327] Consistent with this notion, the relative risks for recurrent ICH compared to future ischaemic stroke where not different within each CMBs subgroup - although the patient numbers and the magnitude of the effect sizes were quite low. One could assume that these relative effects of CMB burden on recurrent ICH and ischaemic stroke risk might tip the risk-benefit balance in favour of antithrombotic treatment use in non-CAA ICH patients with less than ten CMBs.

Our meta-analysis has limitations. Some studies had a small sample size, variable follow-up, and few outcome events, leading to wide confidence intervals around risk estimates. In these studies, inception points (i.e. the start of follow-up), and methods of outcome assessment varied. Studies used different imaging parameters, for example echo time, potentially affecting the detection of CMBs,[27] although the prevalence of CMBs was fairly consistent across studies. Furthermore, studies are subject to selection bias since not all ICH patients undergo T2*-GRE, thus probably excluding more severe cases of ICH. However, our findings represent the most up-to-data summary of available data on the topic and can be generalised in ICH survivors who undergo clinical T2*-GRE MRI. Finally, a potentially important limitation is possible confounding due to lack of adjustment for other baseline variables related to future recurrent ICH risk (e.g. antithrombotic drug use, hypertension treatment, ApoE genotype etc.). Nevertheless, all studies showed a consistent direction of association between CMBs and recurrent ICH, even when adjusted for these potential confounders. Furthermore, we did not have pathological confirmation of the small vessel pathology underlying ICH in each cohort and we acknowledge that the Boston criteria for CAA classification have imperfect specificity.[195, 202] Of note, the type of included cohorts and the presumed underlying causes of ICH are in line with the higher prevalence of hypertensive arteriopathy rather than CAA in Asia compared to the United States or Europe.[32] For this reason only one study had concurrent groups of patients with both CAA-related and non-CAA related ICH.[227]

Spontaneous ICH originates from different cerebral small vessel pathologies which have intrinsically distinct prognostic significance for future stroke (including recurrent ICH and ischaemic stroke). Our findings suggest that CMBs on blood-sensitive MRI sequences are helpful in stratifying the risk of recurrent bleeding in ICH survivors. Furthermore, the use of MRI is critical for phenotyping the presumed underlying aetiology of spontaneous ICH, to inform patients and carers, plan clinical services and design clinical trials. The current data support routine neuroimaging in survivors of spontaneous ICH to define the microangiopathy subtype and CMBs burden to inform prognosis and relative risk of future ICH and ischaemic stroke, which could add to risk–benefit analyses for antiplatelet or anticoagulant use. Whether the risk of recurrent ICH and ischaemic stroke after ICH change over time and the specific influence of antithrombotic drugs on outcome, are important questions which remain unanswered. However, in light of the very high risk of recurrence, in the absence of a compelling indication (e.g. recent coronary stenting, non-valvular atrial fibrillation) antithrombotics should not be routinely used in survivors of CAA-related lobar ICH, especially in the presence of lobar CMBs. For non-CAA related ICH survivors, given that the risk of future ischaemic stroke being almost as high as the risk of recurrent ICH, the distribution and burden of CMBs may need to be taken into account in starting antithrombotics. More research is needed to incorporate these data into a more comprehensive bleeding risk model.

Chapter 11 General discussion, future perspectives and challenges

The CAA story is remarkable in that unlike most neurological disorders, the understanding of the underlying pathology preceded by many years the association with clinical syndromes and disease. Part of this long delay might be explained by a much lower prevalence of CAA in the early 20th century,[351] because most of the population at the time did not live long enough to be at risk for developing this age-related disease. As life expectancy increased significantly and effective control of hypertension has been achieved in the current era, CAA has become an increasingly prominent cause of lobar intracerebral haemorrhage and dementia.[34] Despite the tremendous contributions from neuropathology (including modern molecular pathology) towards understating of CAA, there are now compelling reasons for exploring the disease also from a clinical-radiological perspective. First and foremost, the advent of advanced MRI has increased our ability to detect and define the consequences of CAA in routine clinical practise, including more dynamic features of the disease (i.e. evolution of imaging features over time, incidence of new lesions, clinical recurrence etc.) which are in a way "frozen" (like a "snapshot") in neuropathological examination. Furthermore, the growing knowledge of the underlying pathology, biology, genetics, as well as of the neuroimaging and neurological correlates of CAA can be translated into better techniques for diagnosing and ultimately modifying its natural history.

The main objective of this PhD thesis was to gain novel insights into aspects of potential new CAA biomarkers on applied clinical neuroimaging, which are likely to have increasing relevance in the field. This was accomplished by (a) investigating the clinical and radiological spectrum of transient neurological episodes in CAA; (b) exploring the prevalence, associations and prognostic significance of cSS, a new haemorrhagic marker of the disease; (c) examining MRI-visible PVS in the centrum semiovale as a new CAA marker (including pathophysiological features); (d) identifying pathological, neuroimaging and genetic characteristics in haemorrhagic and non-haemorrhagic CAA with the potential to point towards different phenotypes of the disease; and (e) summarising the evidence on how cerebral microbleeds burden, an established imaging marker of cerebral small vessel disease, relates to recurrent symptomatic ICH according to the presumed MRI-defined underlying arteriopathy (CAA vs. non-CAA).

In this chapter, I would like first to focus the discussion on the potential clinical aspects of CAA-related transient focal neurological episodes in the field of cerebrovascular disease in general. Next, I discuss themes related to the other main thesis findings, namely CAA imaging biomarkers. These are discussed both as meaningful markers to measure the disease's consequences in routine clinical practice, but also in the context of current knowledge and recently published consensus papers on outcome markers for clinical trials

in CAA[300] and cerebral small vessels disease imaging standards.[31] Finally, suggestions for future research are given.

11.1 Transient focal neurological episodes in cerebral amyloid angiopathy: looking beyond TIAs

When vascular neurologists or stroke physicians encounter patients with transient focal neurological symptoms, they usually consider at least five diagnoses: transient ischaemic attacks (TIAs), migraine auras, seizures, peripheral nerve compression, or psychogenic disorders.[352] Surveys show that the diagnosis and management of patients with suspected TIAs, especially by primary care physicians and non-neurologists, remains challenging.[353, 354] As recently as 2008, a report suggested that some general practitioners may not refer patients with transient focal neurological symptoms, while others have a much lower threshold for referral to minimise any risk.[355] It is thus clear that the differential diagnosis of TIAs is not straightforward,[356] despite the simple descriptions given in textbooks. Even for experienced vascular neurologists, it is sometimes impossible to be sure if a transient symptom was truly neurological, and even more difficult to be certain if it was of vascular origin. There is no test to prove a diagnosis of TIA in all cases, although longer attacks are associated with diffusion-weighted imaging (DWI)-positive lesions,[357] which are also associated with an increased risk of future ischaemic stroke.[357] However if the symptoms are very brief, brain imaging, including DWI can be normal; moreover, taking the history can be extremely difficult and the inter-observer agreement for the diagnosis of TIA even amongst experienced neurologists is only modest, with kappa values ranging between 0.65 and 0.78.[358-360]

In our study (Chapter 2) we have highlighted this common diagnostic dilemma with reference to the transient focal neurological episodes (TFNE) which are increasingly recognized in the context of CAA; these attacks can resemble TIAs, migraine auras or seizures (although they are often not quite typical of any of them), but are probably more often related to bleeding in the brain (especially cortical superficial siderosis and acute convexity subarachnoid haemorrhage) rather than ischaemia.[361] It is important to note that TNFEs are not always associated with cSS; there have been reports of transient neurological symptoms in the presence of acute subcortical cerebral microbleeds alone.[362] Since the completion of the study presented here there have been further reports from small cases series on the topic,[363-365] which accord with our data. This is a practical point with potentially important implications for patient care, because: (a) appropriate blood-

sensitive MRI scans can detect small areas of intraparenchymal or subarachnoid bleeding to establish the diagnosis of CAA and likely origin of the episodes; and (b) giving antithrombotic drugs in these patients could increase the risk of serious future intracerebral haemorrhage.

There are similarities between the clinical phenomenology of amyloid spells in our study and those of some of Miller Fisher's famous "late-life migraine accompaniments" (especially the non-visual phenomena):[366, 367] *"Typical of migrainous accompaniments are the build-up and migration of visual scintillations, the march of paresthesia, and progression from one accompaniment to another, characteristics that do not occur in thrombosis and embolism"*.[366] Some of Miller Fisher's cases could have been CAA-related, but at that time there was no access to advanced MRI, and cerebral angiograms were used to "exclude" a vascular cause. In our multicentre study, blood-sensitive MRI sequences, including T2*-weighted gradient-recalled echo (T2*-GRE) and susceptibility-weighted imaging (SWI) revealed that TFNE may be caused by haemorrhagic neuroimaging manifestations of CAA, including superficial cortical siderosis, convexity subarachnoid haemorrhage or lobar cerebral microbleeds.[361] Importantly, and in contrast to Miller Fisher's migraine accompaniments, CAA-related TFNE did not have a benign course, but were associated with a high early risk of symptomatic lobar ICH (about 50% at 2-3 months).[361] It is difficult to speculate as to why Miller Fisher's patients with migraine accompaniments had such a benign course compared to "amyloid spells"; perhaps the follow-up available was not very systematic, or some of his patients had a mild underlying disease process not associated with a high risk of bleeding.

The correct recognition of TFNE in CAA has clinical implications which can be considered according to two scenarios: first, a patient with suspected or probable CAA (e.g. a previous lobar intracerebral haemorrhage) who develops a TFNE; and second, a patient who presents with TFNE but without any previous suspicion of CAA. In both of these settings, our recent findings clearly suggest a key role for T2*-GRE MRI (or other blood-sensitive sequences). In the first scenario, where the suspicion of CAA is high appropriate imaging may identify the possible cause of the TFNE, for example focal superficial cortical siderosis or acute convexity subarachnoid haemorrhage (depending on the timing of the MRI),[28, 171, 191, 195, 361, 368] but also possibly small cortical DWI lesions.[73] The identification of these potential mechanisms of TNFE should mandate aggressive treatment of known risk factors for recurrent ICH, with particular attention to blood pressure control and avoidance of antithrombotics. Anticonvulsant medications may also be helpful if the underlying mechanism is suspected to be related to seizure activity or cortical spreading depression.[170] In the second scenario, the use of T2*-GRE or SWI may reveal previously unsuspected CAA by detecting multiple lobar cerebral microbleeds,[35] old silent

lobar ICHs, cortical superficial siderosis or acute convexity subarachnoid haemorrhage.[28, 195, 202] Since in our study TFNE were observed in patients without a history of symptomatic ICH,[361] they may also prove to be a useful diagnostic marker of CAA, potentially allowing diagnosis earlier in its disease course, before symptomatic ICH occurs. A recent neuropathological study identified TIA–like episodes as being significant clinical predictors of severe CAA in persons with pathologically confirmed Alzheimer's disease.[369] As noted by the authors, the prevalence of a history of TIA-like events in their participants with both Alzheimer's disease and advanced CAA on pathology was striking similar to the reported prevalence of the CAA cohort presented in Chapter 2, at 12.5%.[369] We therefore suggest that the performance of MRI, including not only DWI but also blood-sensitive sequences is essential in the investigation of older patients with unexplained TFNE, especially if they are atypical for TIA, recurrent and stereotyped or occur without known risk factors for typical TIAs.

The magnitude of the problem – i.e. how many patients with TFNE associated with CAA are missed, is currently unknown. Some indirect estimates can be extrapolated from studies including patients with relevant clinical and neuroimaging features. A study by Lovelock et al using data from a population-based cohort (Oxford Vascular Study) scanned early with CT and a hospital-based stroke clinic cohort scanned with MRI, found that approximately 5% of consecutive patients presenting with minor stroke have ICH.[370] In the Rotterdam Study population-based cohort, nonspecific transient focal neurological episodes (not typical of TIAs, including visual symptoms and spreading sensory symptoms) were found in 1%, 1.8%, 2%, and 2.9%, of patients aged 55-64 years, 65-74 years, 75-84 years, and 85 years or older respectively.[371] In the population-based Rotterdam Scan Study cortical superficial siderosis was found in 0.7% of elderly individuals (mean age 69.6 years), all of whom had cerebral microbleeds in lobar locations and in close vicinity to the cortical superficial siderosis.[243]

Further large prospective studies with systematic enquiry about TFNE and appropriate brain and detailed standardized vascular imaging are needed, and should ideally be conducted: (a) in stroke services treating patients with CAA (to get detailed clinical descriptions of TFNEs before ICH and assess their association with CAA compared with other types of ICH); and (b) in services assessing suspected TIAs. Although TFNE diagnosed as TIAs are established as a critical predictor of a high early risk of an ischaemic stroke,[372] some TFNE, in conjunction with appropriate neuroimaging signs, may in the future provide an early warning sign and window for prevention of symptomatic ICH. With this rationale in mind, we are further investigating the prevalence, phenomenology and significance of transient focal neurological symptoms before intracerebral haemorrhage

within the context of CROMIS-2 Study II. This is a large UK-based observational case-control study originally designed to investigate genetic associations with spontaneous and oral anticoagulation-related intracerebral haemorrhage. Adult (>18y) patients treated at participating centres with confirmed intracerebral haemorrhage (confirmed on CT or MRI scans) in the past 1 month, with or without a history of anticoagulant use at the time of the haemorrhage are eligible for the study. We have specifically designed a questionnaire to systematically capture any transient focal neurological symptoms before intracerebral haemorrhage and will further investigate their associations with detailed clinical and imaging data, as well as 6-month outcome.

11.2 Developing biomarkers for cerebral amyloid angiopathy (including outcome markers for trials)

As mentioned throughout this thesis, neuroimaging markers have a central role in defining CAA (and cerebral small vessel disease in general), which falls into at least two basic categories: to make accurate diagnosis of CAA (or identify individuals "at risk" of developing the disease) and predict underlying biological and clinical progression (e.g. bleeding risk stratification). Additionally, neuroimaging or other biomarkers can potentially measure treatment efficacy and effects (sometimes termed "outcome markers" or "surrogate markers"). To address all of these requirements in CAA, a combination of different biomarkers will be needed. Emerging data continue to increase the range of CAA markers which currently include clinical events (e.g. symptomatic intracerebral haemorrhage or cognitive impairment), structural neuroimaging brain lesions (both haemorrhagic and non-haemorrhagic, e.g. cerebral microbleeds, cSS, white matter hyperintensities, cerebral microinfarcts or CSO-PVS), alterations of vascular physiology, and direct visualisation of cerebrovascular amyloid using PET-based radioligands.[300]

There are now grounds for optimism that amyloid-modulating therapies, including immune-based therapies (amyloid-β immunisation), may soon be possible in CAA. There are several potential treatment approaches to modify the progression or prevent CAA, including reducing amyloid-β production, enhancing amyloid-β clearance or protecting small vessels from its toxic effects. A candidate agent which might delay or inhibit the progression of CAA is tramiprosate, an ionic compound which binds soluble amyloid-β, and interferes with the amyloid cascade.[373] Tramiprosate has been shown to be a safe treatment option for patients with suspected CAA in a phase 2 study, supporting future efficacy trials.[374] Emerging data from the use of secretases inhibitors or immunization against

amyloid-β in Alzheimer's disease will be invaluable in guiding further efforts for disease modification in CAA. A phase 2 randomized controlled trial is currently ongoing at specialist centres worldwide, in patients with probable CAA, evaluating the safety and efficacy of Ponezumab, a humanized anti-amyloid monoclonal antibody designed to bind and remove the vascular amyloid-β (ClinicalTrials.gov Identifier: NCT01821118). Other potential disease-modifying mechanisms may also be on the horizon: for example in a transgenic mouse model of CAA, researchers found less severe vascular amyloid deposition, and improved neurological function, associated with knockout of function of the CD36 receptor (found on immune and endothelial cells), with preservation of an important amyloid-β clearing mechanism involving another receptor, LRP-1.[375] Thus, blocking the binding of amyloid-β to CD36 receptor could be a new human therapeutic target in CAA.

Deciding which disease-modifying treatments are the most promising requires: (a) early-phase trials to show safety, feasibility and efficacy, and (b) late-phase pivotal large scale clinical trials to establish those treatments suitable for medical use.[300] The evaluation and implementation of such treatments for CAA increases the urgency with which biomarkers of the disease are needed. By definition, for therapeutic trials, a perfect CAA biomarker should have some additional desirable features: be representative of an established step in disease pathogenesis, correlate well with the underlying pathology or disease severity, and clinical consequences, be efficient for detecting changes in response to treatment, reliably and reproducibly measurable across many trial sites and cost-effective.[300 376] Since there is currently no ideal CAA biomarker with all these desired features, different surrogates should be utilised based on the aim, nature or stage of the trial.

For early-phase proof-of-concept studies aimed at identification of promising candidate treatments, markers with high statistical efficiency for small or short studies should be utilised. These are unlikely to include clinical events or most of the structural neuroimaging CAA markers, as they seem to be more prevalent in only selected patients with advanced disease and might not accumulate at high enough rates to be sensitive enough and practical to capture disease progression over short time periods.[300] For example, symptomatic intracerebral haemorrhage is somewhat more common in individuals with multiple cerebral microbleeds as indicated by our findings in Chapter 10 and is likely a more relevant outcome marker as a recurrent event in the subgroup of patients surviving one or more lobar intracerebral haemorrhage (9.96%; 95% CI: 5.08-14.84 per year in the meta-analysis presented here). In addition, symptomatic lobar intracerebral haemorrhage and most CAA MRI markers probably represent only the "tip of the iceberg" of CAA-related brain structural damage, as they come very late in the postulated CAA pathophysiological pathways, from vascular dysfunction to vascular-mediated brain

parenchyma injury and clinical dysfunction. Hence, quantitative measures of CAA-related abnormalities on the vessels themselves or their function might have a clearer potential to directly detect more subtle (and potentially reversible) effects of the disease in early-phase studies, although there are big challenges to overcome in running these across multiple sites.[300] Findings from recent studies suggest that a physiological biomarker for direct CAA-related vascular injury can be altered cerebrovascular reactivity,[377] measured by the functional MRI (fMRI) blood oxygen level-dependent (BOLD) response to visual stimulation.[378-380] These cross-sectional studies have shown that patients with CAA have reduced and delayed vascular reactivity (lower peak amplitude, longer time to peak and longer time to return to baseline) compared with age-matched healthy controls,[378, 379] and correlate with established markers of disease severity in CAA.[246, 247] Based on these results highlighting the validity of fMRI as a physiologic biomarker of vascular dysfunction,[380] change in cerebrovascular reactivity as measured by the slope (amplitude over time to peak) from visual task-evoked BOLD fMRI was recently adopted as a surrogate endpoint measure in the phase 2 monoclonal antibody study mentioned above (NCT01821118).

Notwithstanding their limitations, compared to physiological biomarkers, clinical events and haemorrhagic or non-haemorrhagic structural neuroimaging biomarkers of CAA are probably more clinically meaningful for use in everyday clinical practise, and alone or in combination are better suited to serve as surrogate markers in late-phase large scale clinical trials. In addition to disease-modifying trials, imaging markers of CAA can be useful as surrogate markers in other interventions, e.g. trials of acute blood pressure lowering in patients with symptomatic ICH. Alternatively, they can be markers affecting prognosis in specific treatment settings, e.g. intravenous thrombolysis for acute ischaemic stroke in the presence of microbleeds.[381]

11.2.1 Cerebral microbleeds, macrobleeds and their inter-relation

Histopathological correlation studies suggest that radiologically-defined microbleeds generally correlate with small collections of blood-breakdown products, which have presumably leaked from fragile small vessels into the brain parenchyma.[172, 330] Hence, cerebral microbleeds differ from other MRI markers of small vessel disease (e.g. lacunes and white matter hyperintensities), in that they seem to provide more direct evidence of microvascular leakiness.[27] As such, cerebral microbleeds in some populations and disease settings might have clinical value as markers of increased future risk of symptomatic ICH

("macrobleeding"), potentially raising clinical dilemmas[27, 382-384] especially regarding the safety of antithrombotic drugs, a cornerstone of stroke prevention treatment.

The two sporadic small vessel pathologies which give rise to both microbleeds and macrobleeds, namely CAA and hypertensive arteriopathy, have distinct natural history and prognosis. As outlined in Chapter 10, the risk of recurrent bleeding after symptomatic intracerebral haemorrhage seems to be significantly higher for CAA-related lobar intracerebral haemorrhage.[37, 149] Lobar cerebral microbleeds, suggesting CAA may thus be a stronger risk factor for future intracerebral haemorrhage than deep cerebral microbleeds. Furthermore, there is still a controversy about the origin of deep (basal ganglia) cerebral microbleeds, with a recent pathological study suggesting an association with ischaemia rather than small haemorrhages.[385] It is of course important to bear in mind that CAA and hypertensive arteriopathy can co-exist and interact, particularly in older populations, and the interpretation of a "mixed" lobar and deep distribution of cerebral microbleeds presents a challenge.

In a cross-sectional case-case analysis, cerebral microbleeds were more commonly found in patients with warfarin-related intracerebral haemorrhage compared to anticoagulated patients without intracerebral haemorrhage and patients with non-anticoagulant related spontaneous intracerebral haemorrhage.[386] These data suggest that both deep perforating arteries in the basal ganglia and cerebral white matter (affected by hypertensive arteriopathy) and CAA-laden leptomeningeal or cortical small vessels weakened by amyloid-β deposition give rise to ongoing minor self-limiting leaks (i.e. cerebral microbleeds). It seems plausible that, under certain circumstances (e.g. minor head trauma, antithrombotic drugs, increased blood pressure, etc.), cerebral microbleeds might not be effectively "sealed off" and instead expand into a life-threatening intracerebral haemorrhage.[387] Taken together, the associations of cerebral microbleeds with age, specific small vessel pathologies (particularly CAA) and warfarin-related intracerebral haemorrhage, and the dynamic evolution of microbleeds over time, suggest an important link between cerebral microbleeds and future macrobleeding.

Are cerebral microbleeds a prognostic biomarker useful for future ICH risk assessment? Currently, there is no simple "yes" or "no" answer to this question; rather, the answer critically depends on the specific patient population under consideration, as well as cerebral microbleeds location and burden. So, which factors predict the small subgroup of those with cerebral microbleeds at risk of future macrobleeding? In patients with CAA-related lobar ICH, longitudinal studies included in the meta-analysis presented here, show that higher number of lobar microbleeds (especially ≥5 cerebral microbleeds) increases the

risk of future recurrent intracerebral haemorrhage,[227] making cerebral microbleeds a powerful risk predictor in this context. It remains unclear whether the presence of cerebral microbleeds might confer a similar increased risk of recurrence in patients with non-CAA related intracerebral haemorrhage. The small sample sizes and lower stroke recurrence rate among non-CAA intracerebral haemorrhage cohorts limit statistical power for detecting an effect. In ischaemic stroke/TIA patients recent estimates based on all pooled prospective studies showed that cerebral microbleeds presence was consistently associated with an increased risk of symptomatic intracerebral haemorrhage (OR: 8.52; 95%CI: 4.23-17.18).[388] This intracerebral haemorrhage risk associated with cerebral microbleeds was higher among Asian patients compared to Caucasians (OR: 10.4 vs. OR: 3.9 respectively).[388] The presence of cerebral microbleeds is also related with an increased risk of future ischaemic stroke in these populations in whom antithrombotic therapy is commonly used.

Another key question is whether microbleeds indicate an increased risk of ICH in persons free of clinical stroke. Two prospective population-based studies found that cerebral microbleeds are associated with both future intracerebral haemorrhage and ischaemic stroke, even in healthy elderly individuals.[389, 390] The number of outcome events was low in these studies, resulting in wide confidence intervals, and the populations were Asian, where the predominant microangiopathy is probably hypertension rather than CAA-related, by contrast with Western populations. Longitudinal data from the Rotterdam scan study are keenly awaited to shed further light on this topic.

A recent study compared the risk of future symptomatic intracerebral haemorrhage in patients who presented with strictly lobar microbleeds without a history of intracerebral haemorrhage (cerebral microbleeds-only CAA) to patients who presented with CAA-related lobar intracerebral haemorrhage.[322] These patients presenting with just lobar cerebral microbleeds on MRI had a clinical, genetic, and neuroimaging profile suggestive of severe underlying CAA pathology. In survival analysis the cerebral microbleeds-only group had a considerable risk of incident intracerebral haemorrhage, although lower compared to the lobar intracerebral haemorrhage cohort. The use of warfarin in a small subgroup of cerebral microbleeds-only CAA patients was associated with a significantly increased risk of future intracerebral haemorrhage after adjusting for other confounders.[322]

A key question is whether cerebral microbleeds are a useful tool for stratifying the risk of ICH prior to deciding on antithrombotic or thrombolytic treatments in these patients. Unfortunately, good quality prospective data in large cohorts are not yet available, particularly regarding thrombolysis.[348] Two meta-analyses[391, 392] highlighted a trend of increased hazard of post-thrombolysis symptomatic intracerebral haemorrhage in ischaemic

stroke patients with cerebral microbleeds (RR: 1.90; 95% CI: 0.92 to 3.93; p=0.082).[269] CAA may be a particular risk factor for post-thrombolysis intracerebral haemorrhages, which are often lobar, multiple and occur remote from the infarct in around 20% of patients.[393] Pathological studies also support a role of pre-existing CAA: one study reported that 70% of patients with thrombolysis-related intracerebral haemorrhage had autopsy-proven CAA compared to 22% of unselected ICH patients of similar age.[394] Finally, an increased risk of ICH was associated with intravenous thrombolysis in CAA transgenic mice.[392, 395, 396] However, more robust data from large well-designed and adequately powered prospective studies are clearly needed, especially to investigate the role of lobar cerebral microbleeds as a marker of CAA in this context.[381] An update meta-analysis on this topic is underway.

Although lobar cerebral microbleeds have great value as a diagnostic biomarker of CAA and can identify patients with established disease (as part of the Boston criteria), many key questions remain unanswered. The current data support the notion that the nature and severity of the underlying arteriopathies, which carry intrinsically different bleeding risks (i.e. CAA vs. hypertensive arteriopathy), may modify the relationship between cerebral microbleeds and macrobleeds. More well-designed large scale prospective studies are needed to draw solid conclusions across different populations (e.g. the CROMIS-2 study in patients anticoagulated after cardioembolic stroke; www.ucl.ac.uk/cromis-2). Studies should ideally take into account not only cerebral microbleeds presence, but also their number, location and progression as well as other markers of small vessel brain injury.[397] For now, the available evidence suggests that in patients with probable CAA the risk of future intracerebral haemorrhage seems to be substantial, and might aggravated by anticoagulation.

Several studies have shown that cerebral microbleeds dynamically accumulate over time, with the burden (presence and number) of baseline cerebral microbleeds predicting the development of new cerebral microbleeds in both deep and lobar regions.[398-400] In the Rotterdam study, appearance of new lobar cerebral microbleeds was reported in 6.1% of 831 healthy elderly individuals in the general population, rescanned after a mean of 3.4 years from their baseline MRI.[399] In the subset with one or more strictly lobar microbleeds at baseline, possibly reflecting clinically silent CAA, 17.5% developed new lobar microbleeds.[399] In a small study of probable CAA patients with intracerebral haemorrhage (n=34), 50% demonstrated new incident lobar cerebral microbleeds, after a mean of 15.8 months.[180] Although larger systematic studies are need for more accurate estimates, the apparent substantially higher prevalence and incidence of lobar cerebral microbleeds compared to symptomatic intracerebral haemorrhage in CAA, makes them an appealing

outcome marker to track disease progression and show a relative reduction in incident events for trials.[300] One of the main downsides of cerebral microbleeds as an outcome marker is their uncertain relevance as a clinically meaningful outcome in patients that will not go on to develop an intracerebral haemorrhage, or populations with underlying CAA in whom the risk of bleeding is low (e.g. Alzheimer's disease). Although strictly lobar cerebral microbleeds have been independently linked to cognitive impairment in the in the general elderly population[401] as well as in patients with ischaemic stroke,[402] their role in neurological dysfunction is still an area under investigation.

11.2.2 Cortical superficial siderosis

Studies presented in this thesis (Chapter 3 and 4) add to the accumulating evidence that cSS is a central component of the haemorrhagic manifestations of CAA, expanding its clinical-imaging spectrum,[167, 403] with the potential to identify new mechanisms and subtypes of the disease. cSS is very common in CAA being found cSS in 60.5% of patients with histopathologically-proven CAA (n=38; mean age 70 ± 6.4 years) but in none of the controls with histopathologically-proven non-CAA-related ICH (n=22; mean age 54 ± 18 years).[195] The clinical-radiological cohort presented in Chapter 3 further investigated the strength of this association and found cSS in 40% of probable CAA patients, but less than 5% of patients with a 'strictly deep' pattern of ICH;[301] cSS was also detected in 15% of patients with a single lobar ICH or mixed (lobar and deep) haemorrhages but in most of these cases the presence of lobar CMBs was suggesting some degree of CAA pathology.[301] In addition, similar to the previous pathological study,[195] cSS was often distant from sites of lobar haemorrhage and occasionally can occur in the absence of intracerebral haemorrhage.[182, 277 196, 303] These and other observations,[203, 404] reinforce the idea that cSS is a separate bleeding event related to CAA, offering equal evidence to lobar cerebral microbleeds or intracerebral haemorrhage for its presence. One study included cSS in modified Boston criteria for CAA diagnosis, showing a potential improvement in sensitivity,[195] but further validation studies are needed to determine whether disseminated cSS increases the specificity for CAA. In the retrospective imaging study presented here, disseminated cSS (affecting at least 4 sulci) appeared to be more specific than focal cSS for CAA.[301] Interestingly, cSS was not associated with lobar CMBs burden raising the possibility that cSS and CMBs may result from different haemorrhagic mechanisms. The population-based Rotterdam Study in 1,062 non-demented subjects ≥60 years, reported cSS in 7 persons (0.7% prevalence),[315] compared to a much higher prevalence of lobar microbleeds (nearly 14%[175]).

In addition to being a trigger of transient focal neurological symptoms, cSS might have another clinically meaningful role in CAA, as a marker of vulnerability to future lobar intracerebral haemorrhage. This has been suggested by case reports and small case series[191, 237, 260, 405] and more recently in a retrospective cohort of patients with CAA-related cSS.[246] The multicentre cohort study of probable or possible CAA presented in Chapter 3, including those patients without cSS at baseline,[302] provides systematic evidence that cSS is a strong and independed predictor of intracerebral haemorrhage risk in these patients. It remains unclear whether isolated cSS without other features of CAA confers an equality high risk of incident intracerebral haemorrhage, e.g. sufficient enough to avoid antithrombotic treatment. Future studies should explore if cSS is a clinically important feature for haemorrhagic risk assessment in older patients without symptoms, in the context of anticoagulants, including recently approved newer direct oral anticoagulants (dabigatran, rivaroxaban, apixaban, edoxaban).[406-409]

Why a particular CAA-related bleeding event results in cSS, lobar ICH or CMBs remains uncertain. A neuropathological series of six autopsy cases of lobar haematomas caused by CAA showed that multiple leptomeningeal arteries can rupture into the subarachnoid space and the brain parenchyma:[248, 316] all cases had multiple haematomas in the subarachnoid space (mainly in the cerebral sulci), as well as intracerebral haematomas. Each intracerebral haematoma was connected to the subarachnoid haematomas at the depth of cerebral sulci or through the lateral margin of the cortex.[248] These observations might explain why patients with disseminated cSS have the highest risk of ICH;[302] disseminated cSS may reflect numerous and widespread leptomeningeal blood vessels damaged by advanced CAA, providing multiple initiation sites for future ICH.[248, 316]

Since lobar cerebral microbleeds and CAA are associated with Alzheimer's disease,[183] some Alzheimer's disease patients may also be expected to have cSS. cSS, CAA and Alzheimer's disease were described on autopsy in two patients with dementia.[410] Two recent imaging studies evaluated cSS in a memory clinic setting.[306, 307] Patients with mild cognitive impairment (MCI) or dementia had prevalence rates of cSS between 2.1% (in MCI) and 7.1% (any dementia according to ICD-10). [72, 73] Patients fulfilling the criteria for Alzheimer's disease showed a prevalence of 4.8%.[31, 32] cSS was associated with lower MMSE score, APOE e4 genotype, higher number of cerebral microbleeds, and white matter hyperintensities burden. The majority of cSS-affected patients did not have a history of ICH. Moreover, not all patients with cSS had cerebral microbleeds, so cSS might be the only diagnostic clue to CAA in these patients. The available data thus support the hypothesis that cSS is a manifestation of CAA in memory clinic cohorts. Although cSS has been related to lower MMSE scores, a direct effect of cSS on cognition has currently not been

convincingly shown. The exact relation of cSS, CAA and Alzheimer's disease requires further study. An interesting hypothesis is that patients with Alzheimer's disease and cSS might have a distinct clinical phenotype (e.g. worse cognitive outcome, high ApoE e4 or e2 genotype frequency, increased ICH risk etc.). cSS could have implications for disease-modifying immunotherapy treatments in Alzheimer's disease for which there are concerns regarding inflammatory and haemorrhagic complications (collectively referred to as amyloid-related imaging abnormalities: ARIA) attributed to rapid amyloid–β shifts from brain parenchyma to the perivascular spaces surrounding the small vessels.[261, 411-413]

cSS holds promise and should be investigated as a potential biomarker (including a marker for ICH risk) in future disease-modification trials in CAA. Similar to lobar cerebral microbleeds, it is tempting to think that the progression of cSS, representing a new bleeding event, can thus be an attractive candidate CAA biomarker, especially if new lesions accumulate at a higher rate compared to cerebral microbleeds or intracerebral haemorrhage (Figure 11.1). However, this topic has not yet been systematically studied.

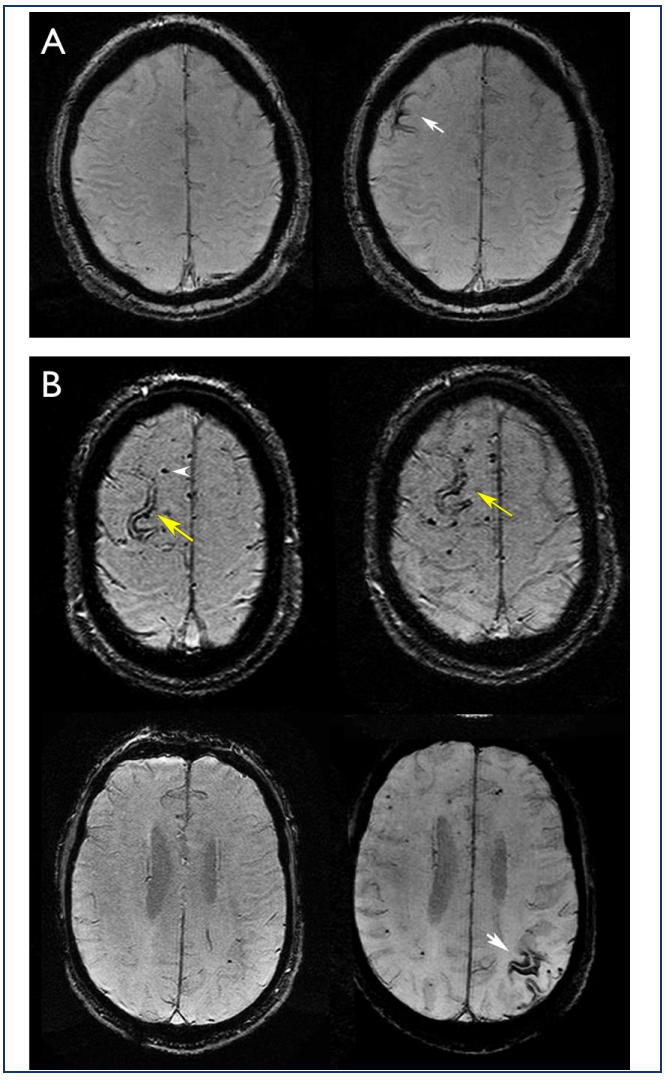

Figure 11.1 Incident and progression of cSS in healthy elderly individuals. A. Baseline (left) and 3-year follow up (right) T2*-GRE scans of the same participant, showing incident cSS (arrow) on the follow up scan. B. Baseline (left) and 3-year follow-up (right) T2*-GRE images of community dwelling subject showing right frontal lobe cSS on baseline not changing at follow up (yellow arrows) as well as new left parietal cSS at follow-up (white arrow). Note the presence of multiple microbleeds in close vicinity to the area cSS (Panel B, white arrowhead). Images provided by Dr Meike W Vernooij.

Table 11.1 Recommended criteria for identification of CAA-related cortical superficial siderosis and acute convexity subarachnoid haemorrhage (acute cSAH)

- Well-defined, homogeneous hypointense curvilinear signal intensity (black) on T2*-GRE or SWI MRI in the superficial layers of the cerebral cortex, within the subarachnoid space, or both
- Blooming effect on T2*-GRE and SWI compared to T1- or T2-weighted sequences
- If there is corresponding signal hyperintensity in the subarachnoid space on proton density-weighted or FLAIR sequences (or hyperdense on CT if available) we recommend the term "acute cSAH"
- Axial T1-weighted or FLAIR images should be used for anatomical confirmation of the gyral location of the signal hypointensities identified on T2*-GRE or SWI sequences
- Absence of infratentorial (brainstem, cerebellum, spinal cord) siderosis
- Ensure exclusion of potential haemorrhagic and non-haemorrhagic mimics (e.g. vessels flow voids, thrombosed vessels, petechial haemorrhagic transformation of infarcts, calcium deposits)
- Consider all potential non-CAA secondary aetiologies of cSS and acute cSAH

Table 11.2 Advised standards for evaluating cortical superficial siderosis (cSS) in research studies of small vessel disease and cerebral amyloid angiopathy

- cSS and acute cSAH should be clearly defined according to the criteria in Panel 1
- cSS should be categorised as focal or disseminated (e.g. in line with modified Boston criteria)
- In each patient the location (cerebral lobes etc.) of cSS and number of cerebral sulci affected should be recorded
- cSS and acute cSAH should be evaluated separately as they convey information on the chronicity of bleeding events
- cSS or acute cSAH clearly connected with any lobar intracerebral haemorrhage should be rated separately as they may not provide clear evidence of individual bleeding events distinct from the ICH
- Other relevant vascular neuroimaging lesions both remote from and in close proximity (e.g. up to 1 cm) to cSS should be evaluated using established standards, e.g. cerebral microbleeds, acute small DWI lesions etc.

Another drawback of cSS as a trial outcome is the lack of standardised rating methods. Since this is a topic not covered in the STRIVE position paper[31] we suggest standards for cSS definition, detection and evaluation as a starting point for future research studies on the topic that will help facilitate cross-study comparisons and pooling of data in collaborative efforts (Table 11.1 and Table 11.2). Interest is increasing in the clinical relevance of cSS and acute convexity subarachnoid haemorrhage: their influence on clinical decision making, future haemorrhage risk and how this is modulated by antithrombotic drug use, their relation with other signs of small-vessel disease, and their independent contribution to cognitive impairment and neurological dysfunction are future research goals. The on-going SuSPect-CAA trial (Superficial Siderosis in Patients with suspected Cerebral Amyloid Angiopathy; clinical trials identifier: NCT01856699) is a multi-centre

study, which aims to prospectively evaluate acute cSAHs and cSS as independent predictors for future stroke and mortality in patients with possible or probable CAA, as well as to better define their clinical presentation and outcome. Data from the SuSPect-CAA study may help define the role of cSS as a neuroimaging biomarker in future CAA treatment trials.[300] Further promising areas of research include correlations of cSS with non-invasive amyloid-β molecular imaging with PET agents such as Pittsburgh compound B (PiB) to determine its relationship to amyloid deposition,[285, 289] assessment of associations with ApoE genotype,[319] and systematic radiological-pathological correlation studies. These studies should shed further light on whether cSS identifies distinct phenotypes of CAA or Alzheimer's disease.

11.2.3 Non-haemorrhagic CAA markers

MRI-visible perivascular spaces

Perivascular spaces (also termed Virchow-Robin spaces) visible on MRI represent one of the most recent, and perhaps less well known, candidate non-haemorrhagic biomarkers of CAA. One of the most important advances in the field was the inclusion of perivascular spaces as a neuroimaging feature of small vessel disease in the STRIVE consensus paper,[31] based on recent data. Here, using a portfolio of hypothesis-driven studies (Chapters 5-8) direct and robust evidence is provided that the topography of perivascular spaces may differ according to the underlying arteriopathy, with CAA being preferentially associated with high numbers of visible perivascular spaces in the centrum semiovale. In summary, the main new findings presented are that: (a) perivascular spaces are very common in spontaneous intracerebral haemorrhage; and (b) different mechanisms may account for perivascular spaces according to their anatomical distribution in intracerebral haemorrhage, consistent with the known localisation of the underlying pathology. Severe centrum semiovale perivascular spaces are more prevalent in patients with lobar intracerebral haemorrhage (probable or possible CAA) and are associated with age, supporting the idea of being a promising new imaging marker of CAA. By contrast, the severity of basal ganglia perivascular spaces is associated with markers of hypertensive arteriopathy suggesting common pathophysiological mechanisms. (c) Severe centrum semiovale perivascular spaces on MRI are present in 85.7% of patients with pathologically proven CAA but in none of the control subjects with intracerebral haemorrhage but no pathological evidence of CAA. Adding centrum semiovale perivascular spaces to current diagnostic criteria might increase the sensitivity, without reducing specificity hence contribute to improving the *in vivo* diagnosis of CAA. (d) MRI-visible perivascular spaces in the centrum semiovale are

associated with PET-based cerebrovascular amyloid-β burden, providing important pathophysiological clues, and further highlighting their possible utility as a surrogate for vascular amyloid burden.

Looking back in light of these findings, previous studies across different populations have highlighted strong associations between basal ganglia perivascular spaces severity (more consistently than centrum semiovale perivascular spaces) and hypertension, white matter hyperintensities and lacunes, which are all typical neuroimaging features of hypertensive arteriopathy.[30, 270, 275] By contrast, centrum semiovale perivascular spaces were not associated with conventional vascular risk factors, but only with increasing age,[30, 272] so may relate to a different underlying small vessel arteriopathy. This is supported by a recent study among memory clinic patients, showing an association between centrum semiovale perivascular spaces and lobar cerebral microbleeds count and basal ganglia perivascular spaces and hypertension.[274] The new observations presented in this thesis are in line with these previous studies, but provide more direct evidence pertinent to CAA.

The link between centrum semiovale perivascular spaces on MRI and CAA is particularly attractive as it may specifically reflect a central and probably early event in the pathophysiology of the disease: interstitial fluid drainage impairment within the perivascular spaces, caused by cumulative leptomeningeal and superficial cortical vascular amyloid-β deposition.[274, 276, 299] Data from molecular pathology studies and animal models support the hypothesis that in CAA, amyloid-β could reach the vessel walls through perivascular spaces, eventually aggregating within them and causing retrograde perivascular spaces due to interstitial fluid blockage, setting in motion a vicious cycle which promotes further amyloid deposition.[276, 282] However, the exact mechanisms of perivascular space enlargement and their appearance on MRI remain poorly understood. The very close anatomical relationship of this perivascular compartment to the perforating small vessels (as recently demonstrated in a 7-Tesla study[414]) suggests that potentially any small vessel pathology could lead to dysfunction and morphological changes of the perivascular compartment (e.g. enlargement),[263] making them visible on conventional neuroimaging.

From the perspective of a pathologist, evidence from autopsy studies to indicate that the signal changes seen on imaging are actually expansion of the perivascular spaces is still very limited.[268, 273] More direct correlation studies between MRI-visible perivascular spaces and underlying pathology-morphology in the setting of CAA are needed if they are to be assessed as a potential biomarker. In addition, as discussed in the relevant Chapters, perivascular spaces will need analysis for their spatial and temporal relation to other markers of CAA, incident appearance over time, and improved protocols for detecting and

quantifying them, ideally using volumetric semi-automated methods.[415] Whether the presence of several visible perivascular spaces is clinically significant also remains controversial, although some studies have associated enlarged perivascular spaces with worse cognitive function.[267]

White matter hyperintensities

White matter hyperintensities of presumed vascular origin are another very common non-haemorrhagic manifestation of CAA on MRI. To date, voxel-based analyses have not definitely shown different patterns of white matter hyperintensities between patients with CAA and hypertensive arteriopathy.[158, 179, 186] Based on the almost inverse topography of CAA and hypertensive arteriopathy in the brain, one would expect striking differences in the regional distribution of white matter hyperintensities (similar to lobar vs. deep cerebral microbleeds, or perivascular spaces) between these small vessel diseases.[186] Surprisingly, some studies suggest no major differences in overall white matter hyperintensities topography in CAA compared to hypertensive arteriopathy.[158, 179] This relatively preserved anatomic distribution despite the specific topography across small vessel diseases, with greatest periventricular lesion burden around the frontal and occipital horns, indicates that white matter injury might occur through chronic microvascular ischaemia in the most vulnerable regions.[186] Despite the lack of pathological specificity for white matter hyperintensities,[24] amyloid-related alterations in vascular reactivity observed in CAA, are consistent with this hypothesis.[377, 378] A more recent study using a semi-quantitative visual scale suggested that, compared to normal elderly controls, patients with CAA-related lobar intracerebral haemorrhage may have a higher prevalence of occipital-predominant (rather than anterior) white matter hyperintensities.[187] Although this finding is interesting and consistent with the relative predilection of CAA vascular pathology for posterior cortical regions,[109] it requires further investigation. A recent study developed a quantitative method to measure the antero-posterior distribution of white matter hyperintensities and provided further lines of evidence that their distribution may indeed represent a promising neuroimaging marker of CAA, at least in specific populations without ICH.[416] In a cohort of patients without intracerebral haemorrhage (n=59) and with pathological evaluation, more posterior white matter hyperintensities distribution was an independent predictor of pathology evidence of CAA, even in cases without lobar microbleeds.[416] Also, in a separate memory clinical cohort (n=259) it was shown that strictly lobar cerebral microbleeds were associated with more posterior white matter hyperintensities distribution after adjusting for other confounders.[416]

An easy and clinically meaningful visual assessment tool of the frontal-to-occipital white matter hyperintensities distribution in patients with different microangiopathies has not yet been established, and a quantitative method might not be practical or sufficient for routine clinical use as a diagnostic biomarker. However, patients with advanced CAA have a larger overall load (i.e. volume) of chronic microvascular white matter hyperintensities compared to healthy elderly people[158] or patients with Alzheimer's disease alone.[284, 417] PET-based cerebrovascular amyloid load is also strongly correlated with ischaemic white matter hyperintensities in CAA.[284] Although data are limited, CAA patients seem to have a high pace of white matter hyperintensities progression[189] Furthermore, damage to white matter in CAA is associated with cognitive impairment independent of the effects of haemorrhage in the brain,[188, 418] making white matter hyperintensities progression a reasonable biomarker to measure a clinically meaningful aspect of CAA-related brain injury.[300] In various other studies (including randomised trials) in the field of small vessel disease, this model has been successfully implemented.[2, 419] Whether a reduction in white matter hyperintensities progression burden is associated with a decrease in the incidence of cognitive and functional decline in CAA needs to be further tested.

Cerebral microinfarcts

Another non-haemorrhagic manifestation of advanced CAA that likely has an important cumulative effect on cognitive function, are cerebral microinfarcts.[197] These have been defined histopathologically as tiny areas of infarction (generally up to a maximum of 1-2 mm), only visible on microscopic tissue examination. Because of their small size, they are typically "invisible" in clinical-radiological studies that rely on conventional structural MRI. Recently, two approaches have detected microinfarcts in vivo (likely at the large end their size spectrum). The first approach is the detection of clinically silent small areas of restricted diffusion on DWI MRI, thought to represent acute microinfarcts, typically described in CAA-related intracerebral haemorrhage cohorts at a frequency of about 20%.[46, 73, 199, 420] The transient appearance of DWI lesions, and their occurrence even in the chronic post-intracerebral haemorrhage time window,[199] suggests they may be part of an ongoing active process, they must accumulate frequently (around 8-10 large microinfarcts lesions per person per year – much higher compare to incident lobar microbleeds) and that the lifetime burden of ischaemia in patients with CAA may therefore be substantial. Nevertheless, this very transience nature of DWI lesions makes them hard to be used as meaningful biomarkers. In addition, there is no neuropathological confirmation that these lesions truly represent cerebral microinfarcts; some may represent 'healed or healing'

microbleeds.[421] The second approach for detecting microinfarcts in vivo, utilized ultra-high-field MRI to reveal small permanent, FLAIR hyperintense and T1 hypointense, structural lesions which have been correlated with microinfarcts at post-mortem.[422] A cross-validation of microinfarcts found on 7T MRI with 3T MRI will open the way for more routine detection of these lesions as a biomarker of ongoing damage in CAA. Of course, a key limitation of both approaches is that with current neuroimaging most burden of cerebral microinfarcts is left undetectable.[423]

11.3 The concept of potential cerebral amyloid angiopathy phenotypes: rational and promise for clinically meaningful biomarkers

As we have seen, during the past decades, progress has been made in understanding the pathophysiology and imaging-clinical expression of CAA and cerebral small vessel disease in general, with trials of disease-modifying drugs being the ultimate goal. What has also become evident is that CAA in not a uniform, but rather a very heterogeneous entity. While sporadic CAA is commonly found in the elderly, it is usually mild and clinically silent. Only a fraction of patients with CAA will go on to develop symptomatic intracerebral haemorrhage, despite the fact that many more might have evidence of haemorrhagic manifestations on neuroimaging, such as multiple lobar cerebral microbleeds or cortical superficial siderosis.[28, 37] In other patients, CAA might cause only white matter hyperintensities and cognitive impairment.[416] In a distinct minority of patients, CAA may be revealed by a striking acute inflammatory encephalopathy and nil else (inflammatory CAA).[162] CAA is also very often present in cases of Alzheimer's syndrome. From these and other observations a picture arises where CAA might have a spectrum of distinct phenotypes of neuropathology and neuroimaging manifestations, comorbid conditions and ultimately clinical expression.[167, 403]

Currently, at least two pathological subtypes of CAA have been identified: CAA-type 1, characterised by amyloid in cortical capillaries (with or without involvement of other vessels), and CAA-type 2, where amyloid deposits are restricted to leptomeningeal and cortical arteries, but not capillaries.[38] The APOE ε4 allele is most strongly associated with CAA-type 1, while APOE ε2 is more associated with CAA-type 2.[107] In addition, it is generally accepted that APOE ε4 promotes vascular amyloid deposition, while APOE ε2 promotes structural vasculopathic changes in amyloid-laden vessels, making them prone to rupture and intracerebral bleeding. CAA-type 1 (especially capillary, more than arteriolar

CAA) appears to be more closely associated with parenchymal amyloid deposition in Alzheimer's disease[108] and may cause luminal obstruction in the most severe stages, potentially explaining the low incidence of microbleeds and intracerebral haemorrhage in patients with Alzheimer's disease and the association between microinfarcts, ischaemic white matter hyperintensities and cognitive impairment in CAA.

Thus, APOE genotype probably influences CAA phenotype, but it is likely that this is not the whole story. Although multiple lobar microbleeds are a marker of increased risk of intracerebral haemorrhage, an interesting study in a cohort of consecutive CAA patients (n=46) suggests that cerebral microbleeds arising from CAA-related pathology might be in some respects distinct from macrohaemorrhages, since they have a bimodal, rather than continuous, size distribution on MRI.[198] In a subset of patients who underwent autopsy, those with high microbleeds counts ("microbleeders") had increased wall thickness of amyloid-positive vessels compared to those with relatively low microbleeds counts ("macrobleeders").[198] Surprisingly, cortical superficial siderosis, another CAA haemorrhagic marker associated with high risk of haemorrhage, is not associated with lobar microbleeds as shown in Chapter 3.[301] It is possible that the topography and severity of CAA may affect the relative likelihood of the small vessel pathology resulting in cerebral microbleeds *versus* macrobleeds or cortical superficial siderosis and convexity subarachnoid haemorrhage. For example, heavy vascular amyloid might actually make cerebral microbleeds more likely than macrobleeding, and leptomeningeal *versus* deeper cortical CAA might make siderosis more likely than cerebral microbleeds. It is also important to bear in mind that ICH may result from mechanisms not related to the presence of cerebral microbleeds or siderosis.

It is tempting to speculate that multiple different (endo-)phenotypes of CAA exist. This assumption touches onto the very basic question of what pathophysiological mechanisms determine whether CAA causes intracerebral haemorrhage or other haemorrhagic manifestations *versus* non-haemorrhagic manifestations, or cognitive decline. This question was partly addressed in the study presented in Chapter 9, and although literature to support firm conclusions is not yet available, it suggests that a "phenotype approach" in CAA provides a conceptual model which might allow greater reliance on the clusters of different surrogate biomarkers in future CAA trials.[300] This approach could also strengthen the links between imaging biomarkers and neurological function. CAA is a dynamic, chronically progressive disease and future longitudinal studies should determine whether the described CAA phenotypes represent really distinct entities adhering to their characteristic imaging feature over time. Maybe one interesting study to perform would be to gather large numbers of possible and probable CAA cases as part of an international network and do principal component and cluster analysis, a statistical procedure that

converts a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. This method can potentially allow determining clusters of clinical-radiological manifestations that could be used to determine prospectively if these phenotypes are really distinct.

Another key requirement is the identification of the exact role of CAA in the context of Alzheimer's disease:[320] is amyloid vascular degeneration a silent partner or a key player in the natural history of neurodegeneration? What are the differences and similarities with sporadic CAA and what lessons can we learn from immunotherapeutic trials of Alzheimer's disease that can be applied to the design of CAA trials? For example, CAA is associated with cerebral microinfarcts and given the common association between CAA and Alzheimer pathology, one mechanism contributing to cognitive impairment in Alzheimer's disease patients may thus be widespread microinfarcts, at least in a subgroup of subjects with especially advanced CAA.[157, 305] In turn, the pattern of cognitive impairment seen in advanced sporadic CAA could represent the cumulative effects of the various haemorrhagic and non-haemorrhagic tissue injuries discussed earlier, in combination with coexistent mild Alzheimer's disease pathology. In addition, as shown in Chapter 9, CAA patients with cognitive impairment and Alzheimer's disease pathology with an APOE e2 genotype might be at increased risk of certain haemorrhagic manifestations of small vessel disease, including intracerebral haemorrhage.[83, 424]

11.4 Final conclusions and future perspectives

Although the use of the clinical and MRI biomarkers presented here should play a key role, future studies should aim to provide stronger support and further insights from pathological-imaging correlations, improved imaging modalities and larger study populations as part of multicentre collaborations and international networks. Obviously an urgent need is to improve the sensitivity and specificity of diagnostic criteria for CAA; a large collaborative effort in underway to explore the value of a range of structural imaging makers presented in this thesis as part of modified Boston diagnostic criteria. Another key area of need is the identification of neuroimaging biomarker dynamic progression over time and whether these also capture clinically relevant changes, which may also contribute in identifying patients at an earlier stage of the disease, ideally before the serious complications of symptomatic intracerebral haemorrhage or cognitive impairment has developed. Early identification will clearly provide the best chance of efficacy for future disease-modifying treatments; however, a big challenge is that not all people with CAA will

go on to develop problems. Advanced imaging methods together with improved pathophysiological understanding of CAA-related or in general small vessel disease related degeneration, will validate new promising biomarkers reflecting different steps in CAA pathogenesis in addition to the known haemorrhagic and non-haemorrhagic structural damage and their clinical consequences. New promising biomarkers in CAA include molecular imaging of amyloid in the brain (e.g. using amyloid radioligands, including ^{11}C Pittsburgh Compound B - PiB),[200] quantitative measures of brain structural integrity and cerebral blood flow, such as diffusion tensor imaging and arterial spin labelling respectively, fMRI as well as cerebrospinal fluid measures of amyloid and markers of neurodegeneration or retinal abnormalities. Studies in the near future should systematically investigate how strongly and specifically each of these biomarkers are related to putative CAA and its severely as assessed by known structural brain imaging findings (e.g. cerebral microbleeds, white matter hyperintensities), and to clinical consequences (e.g. cognitive function). The work described in this thesis has begun to increase the range of potential clinical and MRI biomarkers for CAA, and should contribute to the ultimate aims of early diagnosis and disease modification to slow the progression of this common, yet enigmatic and devastating disease.

Appendices

Appendix A: Supplementary material for Chapter 2

Table 1. Electronic database search strategies

No	Search history	Results
Pubmed search strategy		
1	(amyloid angiopathy OR cerebral amyloid angiopathy) AND (seizure OR seizures OR episode OR episodes OR transient OR attack OR attacks OR aura OR TIA OR subarachnoid OR sub-arachnoid OR SAH OR cSAH OR fSAH OR siderosis OR hemosiderosis OR haemosiderosis OR spells)	202
2	Limit to: Publication Date from 1970/01/01	202
Embase search strategy		
1	(amyloid angiopathy or cerebral amyloid angiopathy).tw.	1911
2	Limit 1 to (human and yr="1970 -Current")	1472
3	(seizure or seizures or episode or epidodes or transient or attack or attacks or aura or TIA or subarachnoid or sub-arachnoid or SAH or cSAH or fSAH or siderosis or h?emosiderosis or spells).tw.	522882
4	Limit 3 to (human and yr="1970 -Current")	270996
5	2 and 4	175

Search software used: OvidSP_UI03.04.02.112, SourceID 54875

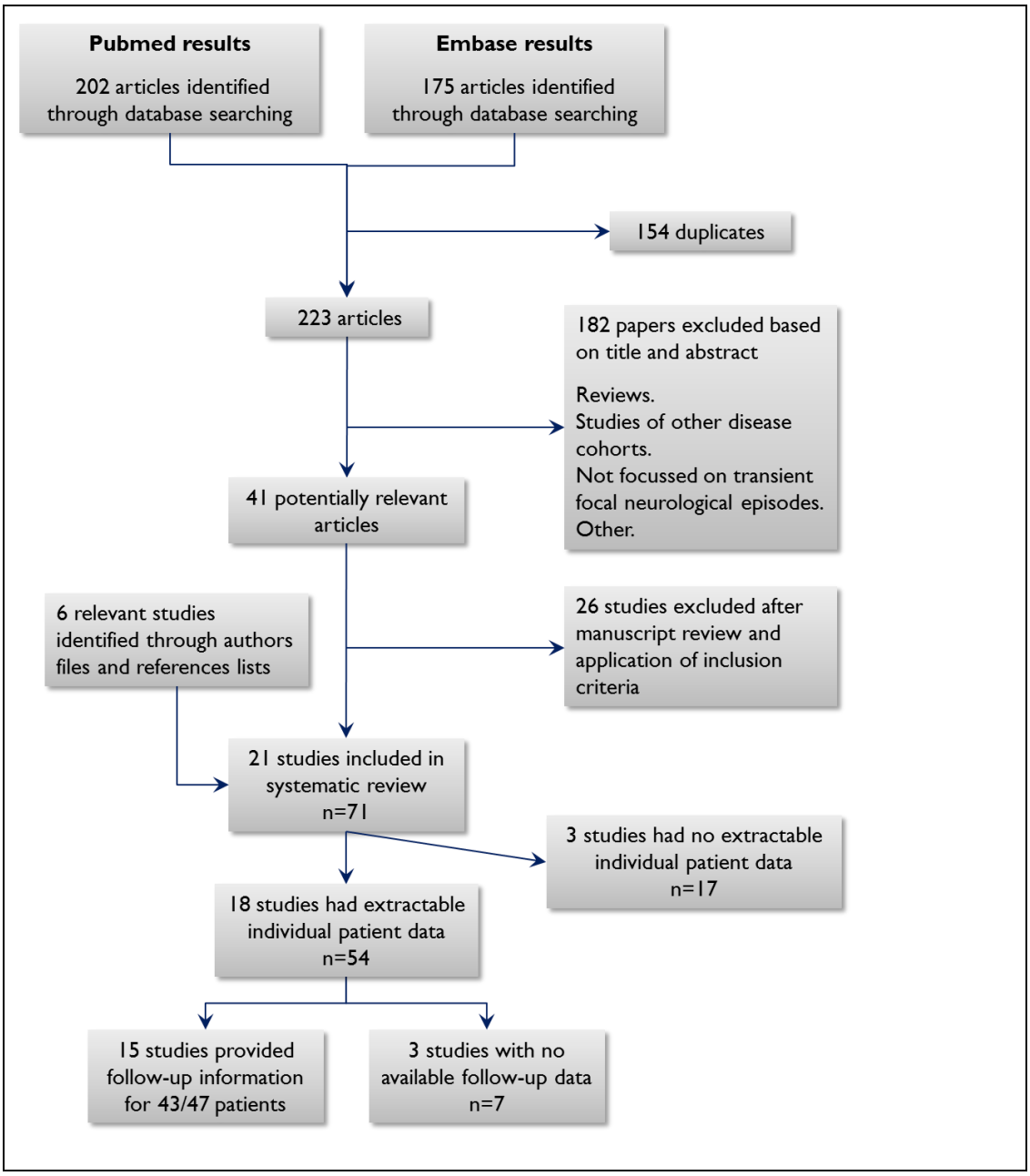

Figure 1. Flow chart of study selection for the systematic review. Twenty-one studies were included in our systematic review.[43, 167-171, 191, 194, 237, 241, 405, 425-434] Three studies had no extractable individual patient data.[43, 191, 194] Fifteen studies (six case series and nine case reports; n=43) had follow-up data.[43, 167, 170, 171, 237, 241, 405, 425, 426, 429-434]

Appendix B: Supplementary material for Chapter 3

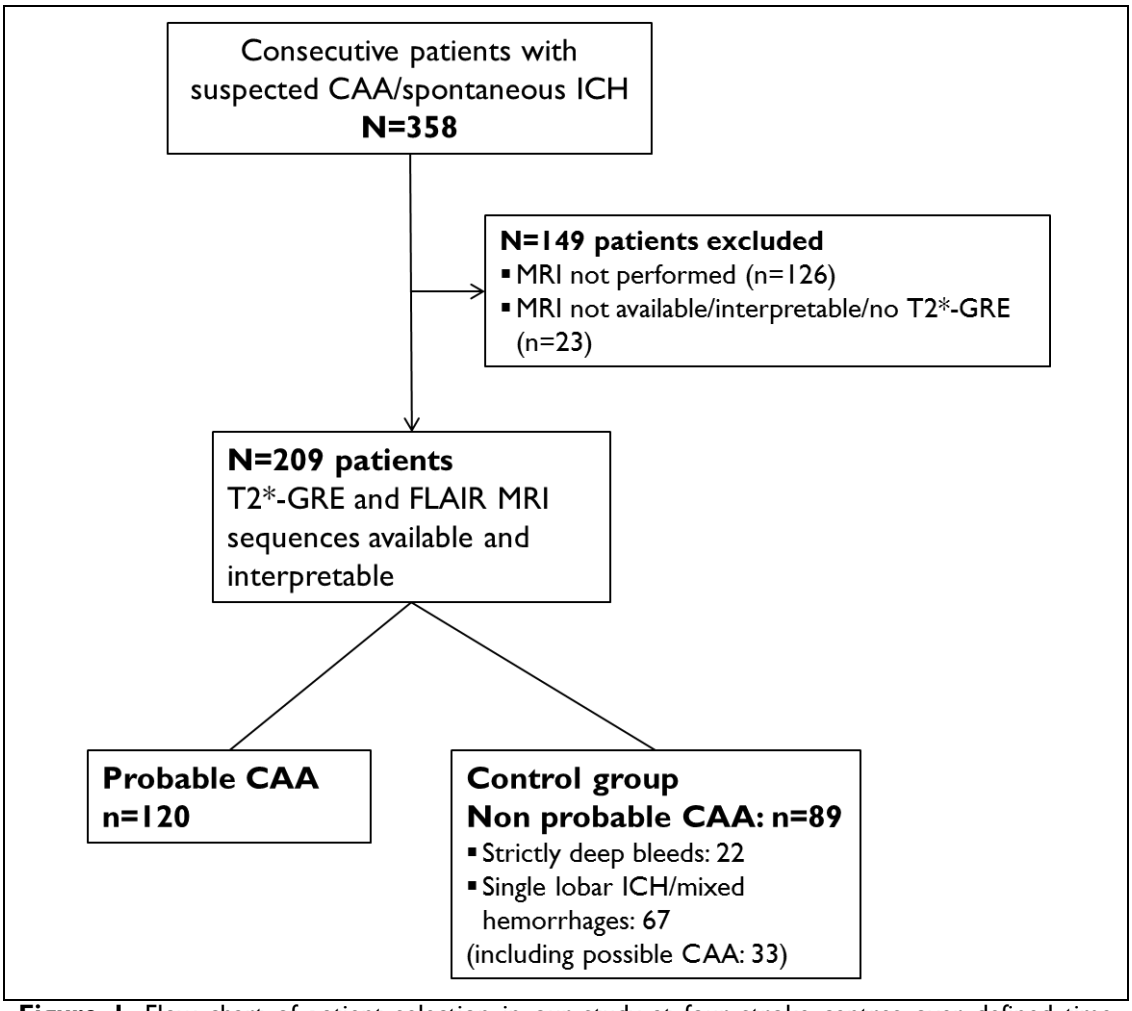

Figure 1. Flow chart of patient selection in our study at four stroke centres over defined time periods. The hospitals were: University College London Hospitals NHS Foundation Trust (London) (03/2003–09/2011), Addenbrooke's Hospital (Cambridge) (07/2002–03/2010), Cliniques Universitaires Saint Luc (Brussels) (12/2003–04/2010) and CHU Mont-Godinne UCL (08/2005–03/2009). Patients excluded from the study were not significantly different from included analysed individuals in median age (71.7 years; IQR: 57.5-76.4 vs. 71.8; IQR: 63.6-77.5, p=0.205), sex (38.4% vs. 39.7% male; p=0.805), and measures of ICH severity including: median (IQR range) ICH volume (10.25 cm^3; 3.2–19.1 cm^3 versus 12.6 cm^3; 3.99–22 cm^3; p=0.621), and median (IQR range) Glasgow Coma Score on admission (15; 12–15 versus 14; 13–15; p=0.987) in patients excluded versus patients included respectively.

Table 1. Frequency of cortical superficial siderosis (cSS) in specific subgroups within the single lobar ICH and mixed haemorrhages comparison group.

Subgroups within the comparison group of patients with single lobar ICH and mixed haemorrhages (n=67)	**Number of cases**	**cSS, n (%)**
Single lobar ICH with no CMBs (i.e. possible CAA by original Boston criteria)	33	2 (6.1%)
Lobar ICH with deep or mixed CMBs	11	2 (18.2%)
Lobar ICH with deep CMBs	2	0 (0)
Lobar ICH with mixed CMBs (lobar and deep)	9	2 (22.2%)
Deep ICH with lobar or mixed CMBs	17	3 (17.7%)
Deep ICH with lobar CMBs	6	1 (16.7%)
Deep ICH with mixed CMBs (lobar and deep)	11	2 (18.2%)
Both lobar and deep ICH, and CMBs	6	3 (50%)

Table 2. Distribution of cerebral microbleeds (CMBs) and intracerebral haemorrhage (ICH) in control patients with single lobar ICH and mixed haemorrhages, and cortical superficial siderosis.

Subject no.	Lobar CMBs	Deep CMBs	Location of ICH
1	7	2	Lobar (2)
2	0	0	Lobar (2); deep (1)
3	23	4	Deep (1)
4	6	1	Lobar (1)
5	16	1	Lobar (2)
6	28	3	Deep (1)
7	1	0	Deep (2) Lobar (1)
8	2	0	Deep (1)
9	0	0	Lobar (1)
10	0	0	Lobar (1)

Table 3. Univariable binary regression analysis testing the factors associated with cortical superficial siderosis in patients with probable cerebral amyloid angiopathy.

	OR (95% CI)	**p-value**
Age (years)	1.06 (1.02-1.11)	0.007
Sex (male)	0.78 (0.37-1.62)	0.500
Hypertension*	0.28 (0.13-0.64)	0.002
On antithrombotics*	0.89 (0.36-2.25)	0.811
History of prior symptomatic ICH (%)*	1.08 (0.50-2.34)	0.845
History of TFNE	6.08 (2.30-16.06)	<0.001
Evidence of chronic lobar ICH	2.49 (1.17-5.31)	0.018
Evidence of acute lobar ICH	1.49 (0.71-3.11)	0.292
Acute ischaemic lesions	1.64 (0.62-4.35)	0.324
Lobar CMBs number	0.97 (0.94-1.01)	0.093
Lobar CMBs (for each category increase)	0.79 (0.55-1.13)	0.201
Leukoaraiosis	0.82 (0.56-1.20)	0.307
Acute cSAH	2.53 (0.84-7.67)	0.099

Appendix C: Supplementary material for Chapter 4

Table 1. Characteristics and comparison of cerebral amyloid angiopathy (CAA) patients with *vs.* without cortical superficial siderosis (cSS). The p-values refer to differences between patients with *vs.* without cSS, using chi-square tests and the Fisher's exact test for categorical variables, and two-sample t-tests or Mann-Whitney U-tests depending on the distribution of continuous variables.

Characteristics	**cSS (+) (n=41)**	**cSS (-) (n=77)**	**p-value**
Age, mean (95% CI), years	73.2 (70.4-76)	70.4 (68.2-72.5)	0.116
Median follow-up time (months) (IQR range)	24.4 (9.5-39.3)	25 (9.2-52.3)	0.527
Sex, male (%)	21 (52.2)	40 (52)	0.940
Hypertension (%)	16 (42.1)	50 (71.4)	0.003**
On antithrombotics at baseline (%)	10 (25)	19 (25.7)	0.937
Previous symptomatic ICH (%) (other than index event)	11 (26.8)	19 (24.7)	0.798
History of TFNE (%)	20 (48.8)	6 (7.8)	<0.001**
Presence of CMBs (%)	31 (75.6)	49 (63.6)	0.185
Number of CMBs, median (IQR range)	4 (1-10)	2 (0-6)	0.110
≥2 CMBs (%)	27 (65.9)	42 (54.6)	0.235
≥5 CMBs (%)	20 (48.8)	21 (27.3)	0.019*
Moderate-to-severe white matter changes (Fazekas score 2 or 3) (%)	16 (40)	36 (47.4)	0.448
Symptomatic lobar ICH at follow-up (%)	14 (34.2)	9 (11.7)	0.003**

*Statistically significant difference at P<0.05; **Statistically significant difference at P<0.01.

Table 2. Different multivariate analysis models of predictors of symptomatic lobar intracerebral haemorrhage (ICH) during follow-up in patients with cerebral amyloid angiopathy. cSS: cortical superficial siderosis; CMBs: cerebral microbleeds.

Model 1: Presence of cSS

Model 1A	**HR (95%CI)**	**p-value**
Presence of cSS	2.27 (0.95-5.44)	0.065
CMBs number	1.02 (0.99-1.05)	0.245
Previous symptomatic ICH	2.01 (0.78-5.17)	0.148
Age (for every year increase)	1.07 (1.02-1.13)	0.011

Model 1B	**HR (95%CI)**	**p-value**
Presence of cSS	2.37 (0.98-5.75)	0.057
Presence of ≥2 CMBs	1.09 (0.44-2.67)	0.857
Previous symptomatic ICH	2.04 (0.80-5.23)	0.138
Age (for every year increase)	1.07 (1.01-1.13)	0.016

Model 1C	**HR (95%CI)**	**p-value**
Presence of cSS	2.50 (1.00-6.18)	0.049
Presence of ≥5 CMBs	0.90 (0.37-2.20)	0.815
Previous symptomatic ICH	2.03 (0.79-5.23)	0.141
Age (for every year increase)	1.07 (1.01-1.13)	0.014

Model 2: Increasing cSS category (from no, to focal and disseminated cSS)

Model 2A	**HR (95%CI)**	**p-value**
Increasing cSS category (from no, to focal and disseminated cSS)	1.72 (1.07-2.75)	0.025
CMBs number	1.02 (0.98-1.05)	0.369
Previous symptomatic ICH	1.96 (0.77-5.04)	0.160
Age (for every year increase)	1.08 (1.02-1.14)	0.010

Model 2B	**HR (95%CI)**	**p-value**
Increasing cSS category (from no, to focal and disseminated cSS)	1.78 (1.11-2.87)	0.018
Presence of ≥2 CMBs	1.03 (0.42-2.53)	0.950
Previous symptomatic ICH	1.97 (0.77-5.05)	0.156
Age (for every year increase)	1.07 (1.01-1.13)	0.015

Model 2C	**HR (95%CI)**	**p-value**
Increasing cSS category (from no, to focal and disseminated cSS)	1.87 (1.14-3.08)	0.014
Presence of ≥5CMBs	0.79 (0.32-1.97)	0.612
Previous symptomatic ICH	1.96 (0.77-5.04)	0.161
Age (for every year increase)	1.07 (1.02-1.13)	0.012

Model 3: Disseminated cSS (vs. all other patients: no and focal cSS)

Model 3A	**HR (95%CI)**	**p-value**
Disseminated cSS (vs. all other patients: no and focal cSS)	2.78 (1.17-6.58)	0.020
CMBs number	1.01 (0.98-1.05)	0.404
Previous symptomatic ICH	1.98 (0.77-5.06)	0.155
Age (for every year increase)	1.08 (1.02-1.15)	0.007

Model 3B	**HR (95%CI)**	**p-value**
Disseminated cSS (vs. all other patients: no and focal cSS)	2.97 (1.26-7.01)	0.013
Presence of ≥2 CMBs	1.05 (0.43-2.58)	0.914
Previous symptomatic ICH	1.99 (0.78-5.07)	0.151
Age (for every year increase)	1.08 (1.02-1.14)	0.010

Model 3C	**HR (95%CI)**	**p-value**
Disseminated cSS (vs. all other patients: no and focal cSS)	3.26 (1.32-8.01)	0.010
Presence of ≥5CMBs	0.79 (0.32-1.98)	0.618
Previous symptomatic ICH	1.99 (0.78-5.10)	0.149
Age (for every year increase)	1.08 (1.02-1.14)	0.008

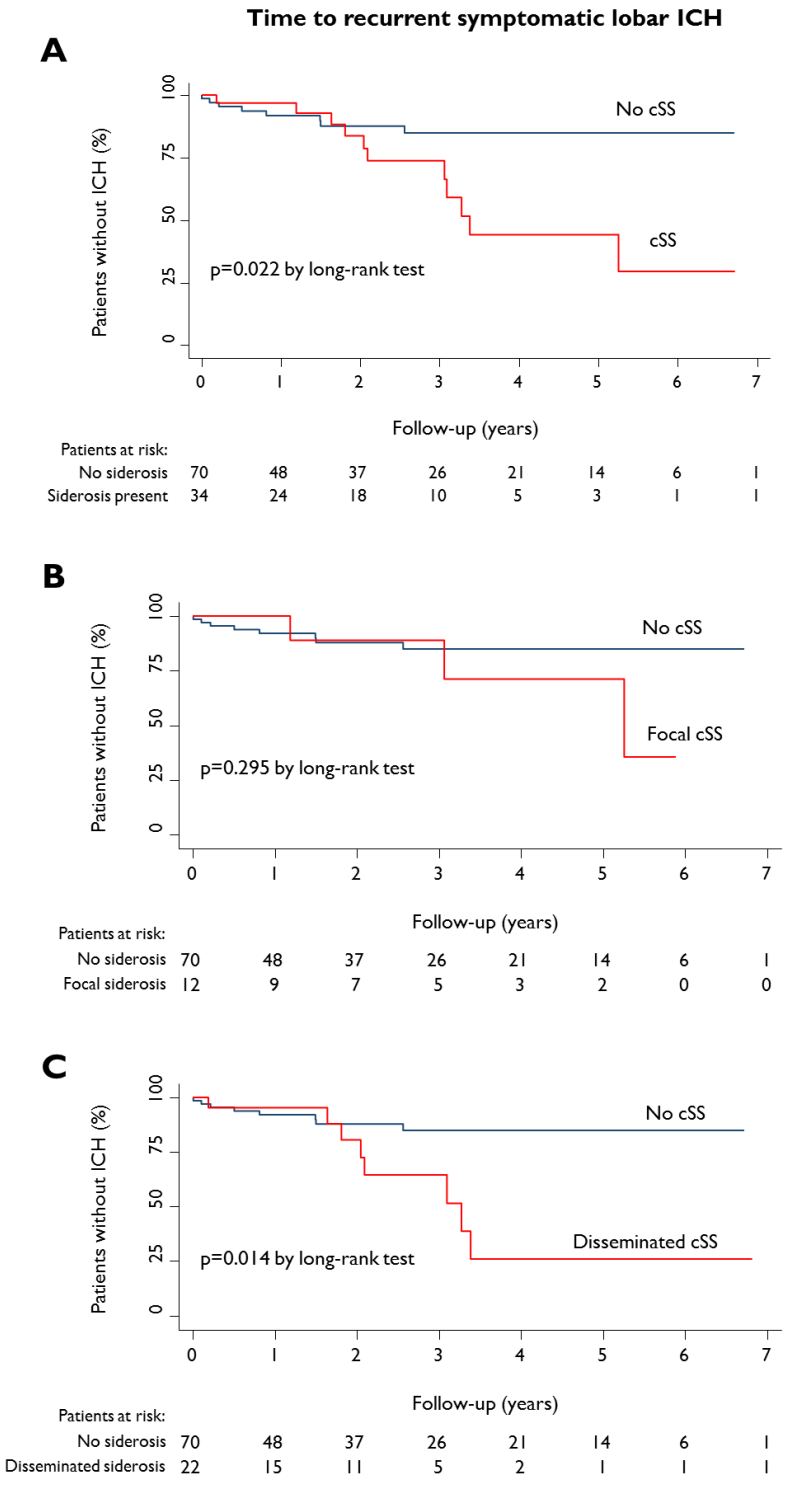

Figure 1. Kaplan-Meier estimates of progression to recurrent symptomatic intracerebral haemorrhage (ICH) in patients with probable or possible cerebral amyloid angiopathy (CAA) and symptomatic lobar ICH at baseline (n=104), in the presence of: (A) cortical superficial siderosis (cSS) (HR: 2.71; 95%CI: 1.12-6.60; p=0.027); (B) focal cortical superficial siderosis (HR: 2.00; 95%CI: 0.53-7.56 p=0.305); and (C) disseminated (>3 sulci) cortical superficial siderosis (HR: 3.18 ; 95%CI: 1.20-8.40; p=0.020). Testing of significance is by the log-rank test.

Appendix D: Supplementary material for Chapter 5

Table 1. Details of basic MR imaging parameters.

University College London Hospitals NHS Foundation Trust, UK MRIs were carried at 1.5T field strength. T2-weighted sequences were acquired in the axial plane with parameters as follows: slice thickness 5 mm, slice gap 1-2 mm, echo time 100–106 ms, repetition time 4763/6000. Axial T2*-GRE images were obtained at: repetition time 300/800 ms, echo time 40/26 ms, slice thickness 5 mm, slice gap 1.5 mm.
Cliniques Universitaires Saint Luc, Brussels, Belgium MRIs were carried at 1.5T field strength. T2-weighted sequences were acquired in the axial plane with parameters as follows: slice thickness 4-5 mm, slice gap 1 mm, echo time 89–90 ms, repetition time 4768/6040. Axial T2*-GRE sequences were acquired, with parameters as follows: repetition time 230–240 ms, echo time 50–70 ms, slice thickness 5 mm, gap 1 mm.
Addenbrooke's Hospital, Cambridge, UK MRIs were carried out at 1.5T field strength. T2-weighted sequences were acquired in the axial plane with parameters as follows: slice thickness 4-5 mm, slice gap 1-3 mm, echo time 80–100 ms. T2*-GRE sequences were obtained in the axial plane using the following parameters: repetition time 460–660 ms, echo time 15 ms, field of view 22 cm, slice thickness 6 mm, slice gap 7 mm.
CHU Mont-Godinne UCL, Belgium MRIs were carried at 1.5T field strength. T2-weighted sequences were acquired in the axial plane with parameters as follows: slice thickness 5 mm, slice gap 1-2 mm, echo time 85–100 ms, repetition time 6000. T2*-GRE sequences were obtained in the axial plane: repetition time 921 ms, echo time 22 ms, slice thickness 4 mm, slice gap 10%.

Table 2. Associations between the severity of centrum semiovale and basal ganglia EPVS. A comparison using chi square test across categories, showed no significant relationship between EPVS severity in the two anatomical areas (p=0.100 for trend).

N (%)	**Centrum semiovale EPVS**				
Basal ganglia EPVS	Mild: 1-10	Moderate: 11-20	Frequent: 21-40	Severe: >40	*Total*
No EPVS	1 (14.29)	0 (0)	1 (2.33)	0 (0)	2 (1.65)
Mild: 1-10	2 (28.57)	23 (63.89)	21 (48.84)	24 (68.57)	70 (57.85)
Moderate: 11-20	2 (28.57)	11 (30.56)	16 (37.21)	8 (22.86)	37 (30.58)
Frequent: 21-40	2 (28.57)	2 (5.56)	5 (11.63)	2 (5.71)	11 (9.09)
Severe: >40	0 (0)	0 (0)	0 (0)	1 (2.86)	1 (0.83)
Total	7 (100)	36 (100)	43 (100)	35 (100)	121 (100)

Appendix E: Supplementary material for Chapter 6

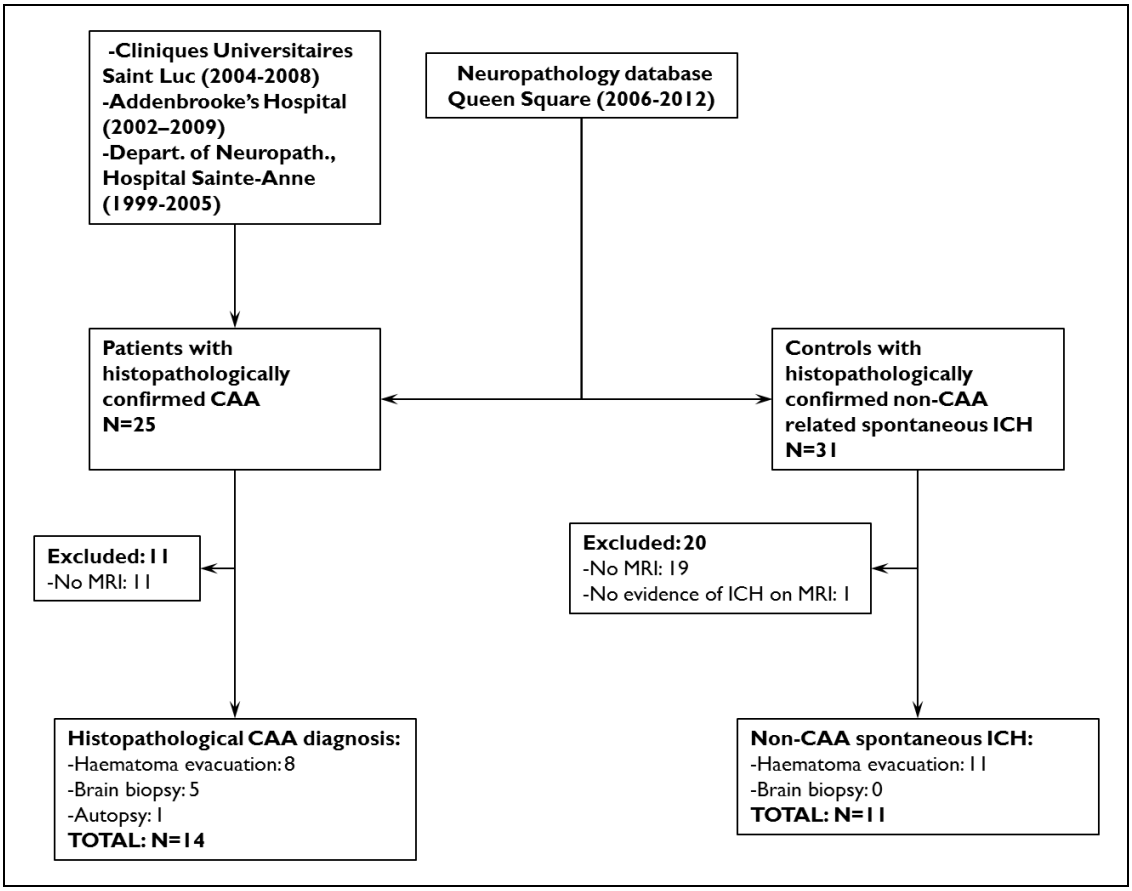

Figure 1. Flow chart: identification of patients with histopathologically confirmed cerebral amyloid angiopathy (CAA) and control patients with non-CAA related spontaneous ICH (ICH). Patients were identified retrospectively through an electronic keyword search of the Neuropathology database in the Division of Neuropathology, Queen Square (2006-2012) and Department of Neuropathology, Hospital Sainte-Anne, Paris, (1999-2005). The electronic keyword searches were performed in the "Microscopic finding", "Diagnosis" and "Comments" database fields of the Neuropathology databases. We also systematically searched prospective clinical databases of consecutive patients with suspected CAA referred to two specialist stroke centres (Cliniques Universitaires Saint Luc-Brussels: 12/2004–04/2008; and Addenbrooke's Hospital-Cambridge: 07/2002–12/2009) to identify additional patients with histopathologically proven CAA (Figure 1). Ten of the CAA patients were admitted for spontaneous lobar ICH, one because of ischaemic stroke, one with transient focal neurological episodes, and four due to cognitive decline. All of the control patients were admitted with spontaneous ICH.

Appendix F: Supplementary material for Chapter 9

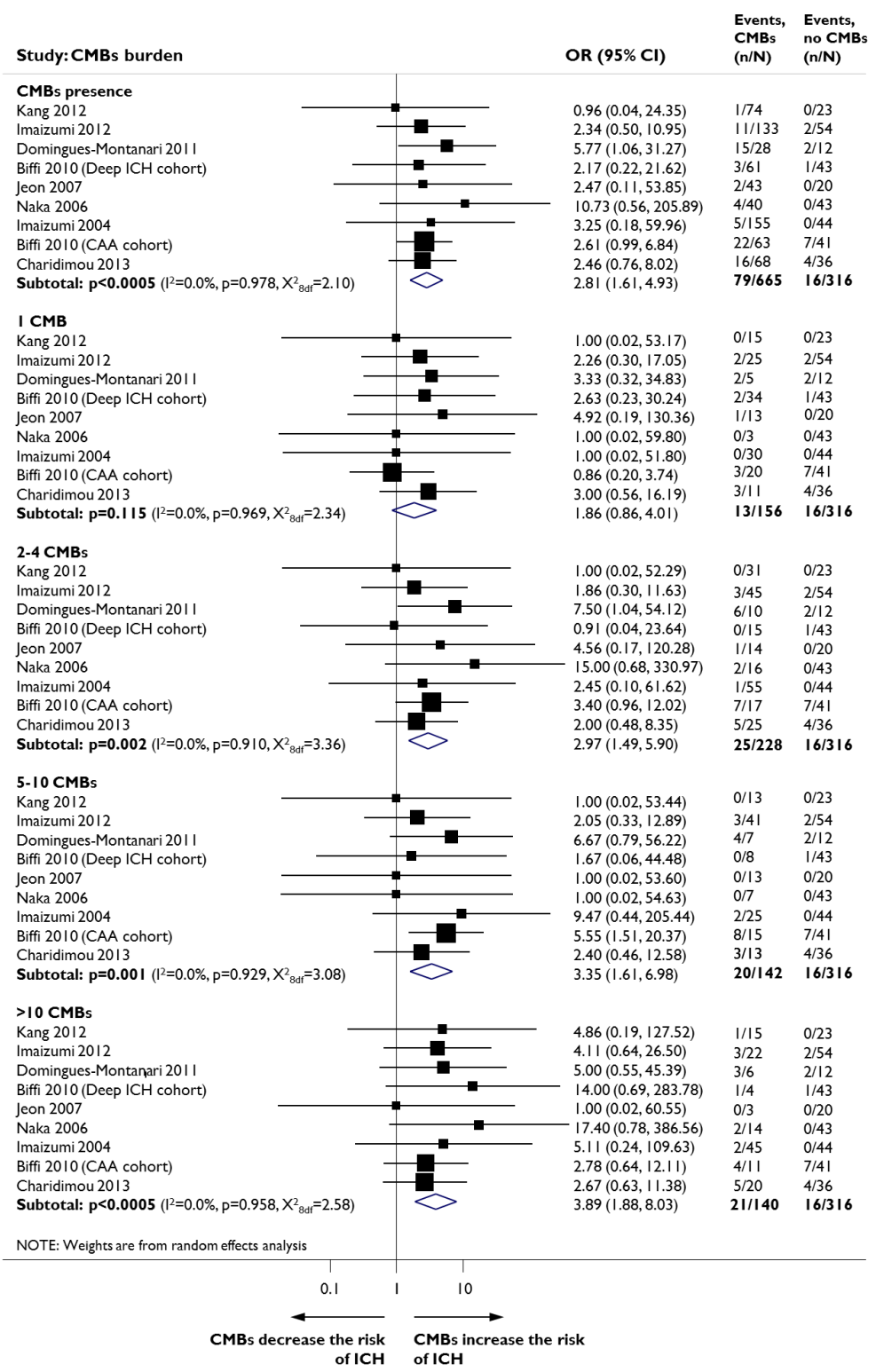

Figure 1. Meta-analysis of effect of cerebral microbleeds (CMBs) presence and burden on the risk of recurrent spontaneous ICH in all the ICH cohorts (CAA and non-CAA).

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