Post mortem assessment in vascular dementia: advances and aspirations

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Abstract

Cerebrovascular lesions are a frequent finding in the elderly population. However, the impact of these lesions on cognitive performance, the prevalence of vascular dementia (VaD) and the pathophysiology behind characteristic *in vivo* imaging findings are subject to controversy. Moreover, there are no standardised criteria for the neuropathological assessment of cerebrovascular disease (CVD) or its related lesions in human *post mortem* brains and conventional histological techniques may indeed be insufficient to fully reflect the consequences of CVD. Here, we review and critically discuss some aspects of CVD including both neuropathological and *in vivo* imaging findings, prevalence rates and clinico-pathological correlations. In addition, we present novel methodologies for the assessment of human *post mortem* brains that should aid to further clarify the impact of CVD on cognitive performance.

Key words; Vascular dementia, vascular cognitive impairment, cerebrovascular disease, cerebrovascular lesions, neuropathology, magnetic resonance imaging, *post mortem* MRI, mixed dementia

Background

Cerebrovascular disease (CVD) is highly prevalent in brains of the elderly. However, its impact on cognition is less clear and while prevalence rates of vascular dementia (VaD) are high in clinical studies, compared to *post mortem* studies, where CVD is rarely found to be the neuropathological correlate of clinical dementia. In this review we highlight some of the current problems in the diagnosis of CVD and present novel approaches that may prove helpful to elucidate the impact of CVD on cognitive performance.

Methods

This article was conceived at the 9th International Congress of Vascular Dementia by participants of the Neuropathology symposium following a discussion on current problems regarding the clinical and pathological diagnosis of VaD and CVD.

Neuropathology of cerebrovascular disease

Degenerative cerebral vessel pathology

Mainly three diseases of cerebral blood vessels can contribute to vascular cognitive impairment and/or vascular dementia (VCI/VaD): *i)* atherosclerosis (AS), *ii)* small vessel disease *(*SVD) and *iii*) cerebral amyloid angiopathy (CAA). AS is a degenerative vessel disorder affecting large to medium sized cerebral arteries, most commonly the basilar artery and the circle of Willis [1], and results in the formation of atherosclerotic plaques due to accumulation of cholesterol-laden macrophages. Mature atherosclerotic plaques calcify, which may lead to narrowing of the artery lumen and are prone to rupture resulting in subsequent thrombosis and potential thrombo-embolism [2]. SVD encompasses three degenerative alterations of the vessels walls of smaller cerebral arteries and arterioles: *i*) SVD-AS has a similar pathogenesis to large vessel AS but affects small intracerebral and leptomeningeal arteries (200-800µm in diameter) developing microatheromas. *ii*) Lipohyalinosis affects smaller arteries and arterioles (40-300µm in diameter) and is characterized by asymmetric fibrosis/hyalinosis associated with cholesterol-laden macrophages infiltration that can occur with or without plasma protein leakage as a result of blood-brain-barrier (BBB) breakdown. *iii*) Arteriolosclerosis presents as concentric hyaline thickening of small arterioles (40- 150µm) that may lead to stenosis of the blood vessel [3]. SVD initially manifests as lipohyalinosis and arteriolosclerosis in vessels of the basal ganglia, i.e., putamen and globus pallidus, then in leptomeningeal arteries. In parallel SVD-AS develops in leptomeningeal arteries, while it affects brain stem arterioles only in end stages of SVD. Cortical vessels on the other hand remain relatively free of SVD pathology [4]. CAA is characterized by the deposition of amyloid-beta (Aβ) (predominately Aβ-40) in the vessel walls of leptomeningeal and cortical arteries, arterioles, capillaries and, rarely, veins [5], resulting in loss of smooth muscle cells and disruption of vessel architecture, and in very severe stages, Aβ depositions in the adjacent surrounding neuropil (i.e. dyshoric changes). Topographically, CAA usually presents in the neocortex, with more frequent and severe deposition seen in the occipital region, followed by the allocortex and cerebellum, and finally, the basal ganglia, thalamus and white matter [6].

Cerebrovascular lesions

AS, SVD and CAA can all lead to various cerebrovascular lesions: *i*) infarcts, *ii*) haemorrhages and *iii*) white matter lesions (WML). Ischaemic infarcts are typically observed after thrombotic or thromboembolic occlusion of large to medium arteries often as the result of an AS plaque rupture. Haemorrhagic infarcts can occur in infarcted regions in which the remaining vessels have fragile vessel walls as a result of SVD or CAA or may be caused by venous obstruction while collateral blood influx into an infarcted area is a less frequent cause for haemorrhagic infarcts in the brain [7]. Large infarcts (>15mm^3) are frequently the result of

thrombotic (AS) or thromboembolic (AS, extracranial AS, cardiogenic) occlusion of the vessels lumen [8]. Lacunar infarcts, i.e., cavitating infarcts (5-15mm^3), are largely confined to the white matter and subcortical grey matter and, hence, primarily associated with SVD [9], whereas, microinfarcts (<5mm in diameter) can be present in both the cortex and white matter and are associated with CAA and SVD respectively [3]. Whilst cerebral haemorrhages (>10mm in diameter) can result from all types of vessel disorders, those located in the subcortical, brain stem and deep white matter are strongly associated with SVD, whist lobar haemorrhages are most commonly associated with CAA. Small haemorrhages (<10mm in diameter) and microbleeds may histologically indeed appear as extravasations of erythrocytes, but more frequently the only histological correlate of microbleeds diagnosed by *in vivo* imaging are haemosiderin-laden macrophages in the perivascular space which may or may not necessarily be the residue of a bleeding. In the cortex, small haemorrhages and microbleeds are associated with CAA [10], while those located in the white matter, subcortical grey matter and brain stem are associated with SVD [11]. WML encompass structural damage histologically characterized by white matter rarefaction, i.e., demyelination and axonal loss, mild astrocytosis, oedema and macrophage reaction [3]. Of note, subcortical U-fibers are usually spared. WML are generally assumed by clinicians and radiologists to be the result of SVD-related chronic hypoperfusion and BBB alterations [12-14], although it is unclear if periventricular (PV)-WML and deep WML share the same pathogenesis (Figure 1). In addition, severe neurodegenerative pathology in the cortex has been recently suggested to cause WML (see section "white matter lesions").

Pathological classifications of vascular dementia

Cerebrovascular lesions can result in ‘pure’ VaD, i.e., extensive vascular lesions, without widespread neurodegenerative pathology, e.g., Alzheimer’s disease (AD) or Lewy body pathology, which explains the clinical dementia. Classification of VaD in three major forms depend on lesion distribution: *i)* multi infarct dementia is characterized by multiple lacunar and microinfarcts, as well as small and/or large infarcts in the cortex and subcortical regions, of which the total amount of damaged cerebral tissue results in a significant decrease in functional brain capacity, surpassing the threshold for cognitive impairment. *ii)* In contrast, strategic infarct dementia is the result of a single infarct in a strategic region of the brain that results in significant cognitive deficits when destroyed, e.g., a single lacunar or micro-infarct in the hippocampus can lead to marked memory impairment [15, 16]. *iii)* Lastly, subcortical vascular encephalopathy (synonymous with Binswanger’s disease) describes confluent severe demyelination and axonal loss in the white matter with sparing of subcortical U-fibers [13, 15, 16]; for review see [17].

Co-morbidity of cerebrovascular disease and Alzheimer's disease pathology

A large proportion of demented subjects that exhibit significant cerebrovascular lesions also exhibit more severe concomitant AD pathology [18], i.e., deposits of hyperphosphorylated tau (HPτ) and Aβ, that fulfills the neuropathological criteria for AD (Braak NFT stage V/VI, CERAD score of 3, and Aβ phase 5 according to the NIA–AA guidelines [19-22]), and is therefore classified as mixed AD/VaD. The distinction between AD, VaD, and mixed AD/VaD remains a controversial issue and poses a difficult challenge (see section 'Clinico-pathological correlations and mismatch in VaD and mixed VaD/AD).

Prevalence of vascular dementia

In clinical population-based series, the prevalence of VaD/VCI averages 8-15.8% (in Japan 23.6-35%) with standardized incidence rates between 0.42 and 2.68 per 1000/year, increasing with age [23]. The range is broader in clinical studies using convenience series from western memory clinics, varying from 4.5-39%. Nevertheless, in none of these cases, the prevalence rates of VaD/VCI are likely accurate because even the best clinical diagnostic criteria show only moderate sensitivity (approximately 50%) and variable specificity (range 64-98%) [23, 24]. VaD in autopsy series also varies tremendously ranging from 0.03-58% [23] and this variation is partly due to the lack of internationally accepted consensus criteria for the neuropathological diagnosis of VaD. In elderly subjects, the prevalence of "pure" VaD ranges from 5-78%. In the oldest-old, i.e., 90 years or over, the rates of "pure" VaD drops (from 4.5-46.8%) but mixed AD/VaD is becoming more prevalent reflecting a constant age-related increase of neurodegenerative changes. Rigorous population-based clinico-pathological correlative studies addressing the prevalence of VaD are few, but are arguably more informative about the actual prevalence of VaD/VCI. In population-based clinico-pathological series, the prevalence of "pure" VaD and mixed AD/VaD ranged from 2.4% to 23.7% and from 4.1% to 21.6%, respectively [25, 26]. The range is still wide and this may reflect regional differences in managing cardiovascular risk factors and ethnic-related genetic variances. In general terms, these studies show that the prevalence of VaD/VCI is higher in developing countries and Japan. For instance, in a clinico-pathological study from Brazil, where cardiovascular risks are poorly managed, the prevalence of "pure" VaD was 21.2%, one of the highest detected in population-based studies [26]. On the other hand, in a retrospective hospital-based study in 1700 consecutive autopsy cases of demented elderly in Vienna, Austria (mean age 84.3±5.4 years; 90% over 70 years) "pure" VaD was observed in 10.7%, decreasing between age 60 and 90+ from 15.0 to 8.7% [27]. VaD and VCI are potentially preventable diseases, and therefore, studies

focusing on its prevalence, incidence and risks factors in the different populations are essential to guide public policies.

Controversies in clinico-pathological correlation of cerebrovascular disease

At present there are two fundamental issues regarding the assessment and diagnosis of VaD. Firstly, there are no currently accepted neuropathological consensus criteria regarding the assessment of VaD, VCI, cerebrovascular pathology or related lesions [28]. Neuropathological assessment of the *post mortem* brain is required to reach a definitive diagnosis and must be carried out in a standardised manner, applying reproducible methods and following generally accepted consensus criteria [29]. Widely used consensus criteria for the pathological diagnosis of common neurodegenerative disease, i.e., AD and Lewy body diseases (LBD) have been available for some time [19-21, 30-33]. However, despite several attempts being made without major success [16, 34-36], generally accepted neuropathological criteria for diagnosing VaD are still unavailable. Secondly, general assumptions regarding the underlying pathology of frequently observed *in vivo* magnetic resonance imaging (MRI) findings might not always be accurate. Neuroimaging is indeed an important tool in the clinical diagnosis of cerebrovascular lesions and imaging-pathological correlative studies are aiming to bridge the gap between *in vivo* imaging and *post mortem* neuropathology. However, general assumptions regarding the underlying pathogenesis of common *in vivo* MRI findings are not unequivocally corroborated by neuropathological findings and this may result in inadequate clinical diagnosis and treatment.

Clinico-pathological correlations and mismatch in VaD and mixed AD/VaD

Various forms of cerebrovascular disorders may lead to cognitive impairment and dementia in the elderly [17]. While 'pure' VaD - being most frequently caused by infarctions - is rare, it is generally assumed that cerebrovascular pathology contributes to the development of cognitive impairment in other neurodegenerative diseases, in particular in mixed AD/VaD. Such mixed disorders are frequently observed in the brains of elderly individuals and their prevalence and severity increase with advancing age [37]. In aged individuals, lacunes, microbleeds, WML and microinfarcts have been associated with cognitive decline, including reduced mental speed and impaired executive functions [38]. Cerebral SVD may interact with pathophysiological processes in AD either independent of each other or due to additive or synergistic effects on cognitive decline [39, 40]. There are several clinical classification criteria for VaD/VCI, such as the NINDS-AIREN criteria, the State of California Disease Diagnostic and Treatment Centers (ADDTC) criteria, the ICS-10 and DSM-V criteria. They distinguish between: possible VaD - clinical criteria of dementia with

focal clinical or imaging signs of one or more infarcts, gait disorder, pseudobulbar palsy, personality and mood changes; probable VaD - all signs of dementia, two or more infarcts followed by dementia and imaging signs of at least one extracerebellar infarct, and, finally, proven VaD- clinically proven dementia and pathological demonstration of multiple cerebrovascular lesions and mixed dementia. The diagnosis of VaD/VCI is reflected by recent clinical criteria [41] that are based on evidence of infarcts, WMH and microbleeds, using structural MRI. Several autopsy studies have demonstrated that microinfarcts are major risks for VCI; however, microinfarcts can not be detected by 1.5 and 3.0 T MRI and the naked eye examination, while they may be seen on novel high-resolution 7.0 T MRI [42-45]. However, no accepted and pathologically validated criteria for the diagnosis of VaD/VCI are currently available [46], therefore, the diagnostic accuracy of possible VaD is still relatively poor with an average sensitivity of 0.49 (range 0.20-0.89) and an average specificity of 0.88 (range 0.64-0.98) [47, 48]. The relative weighting of pathological lesions on longitudinal cognitive decline shows the following rank order significance: NFT > Lewy bodies > Aβ plaques > macroscopic infarcts [49]. The profile of cognitive impairment for neuropathologically defined mixed AD/VaD and SVD resembled that seen in AD cases; memory scores were lower than executive scores by nearly one standard deviation, where all cognitive domains were impaired more or less equally [50]. These findings suggest that, in general, when SVD is combined with AD, the effects of AD on severity and profile of cognitive impairment overwhelm those contributed by SVD. Analyses of longitudinal, clinical, imaging and neuropathological studies confirmed the impact of AD pathology and demonstrate the usefulness of multivariate, continuous approaches to understand brain-behavior relationships, as well as highlighting the limitations of current clinical, neuroimaging and neuropathological measures to model and predict cognitive decline [49]. During the past few years, however, the detection of early AD changes, beginning in preclinical stages of cognitive impairment, became a reality with the inception of amyloid PET tracers and various Aβ ligands, e.g., Pittsburgh Imaging Compound B (PiB), fluorbetapir and flutemetamol [51]. Several studies illustrated how amyloid PET imaging will improve our ability to recognize AD and mixed AD/VaD cases of dementia.

Converging evidence show that VaD and AD exert additive adverse effects on cognitive health. Do vascular risk factors and CVD merely increase the co-occurrence of two separate processes, i.e. AD and silent/symptomatic VCI, which shifts the syndrome diagnosis of dementia and AD earlier; or do both factors potentiate AD-specific pathophysiological pathways? Recent neuroimaging studies in cognitively normal elderly subjects aged 70-90 years suggested that vascular and amyloid pathologies are at least partly

independent predictors of cognitive decline in the elderly and that cognitive reserve seems to offset the deterioration effect of both pathologies on the cognitive trajectories [52].

Concomitant cerebrovascular lesions (CVL) increase the risk and severity of clinical dementia in elderly individuals meeting the neuropathological criteria for AD [53-55]. On the other hand, many studies emphasize additional pathogenesis in older non-demented people, in particular CVLs, e.g., small or large cerebral infarcts, lacunar and WMLs in 22 up to almost 100% [48, 55-61]. Cerebral infarcts were seen in 21-48% of non-demented seniors, with a higher frequency of large infarcts [48, 55, 58, 60, 62-64], and of CAA [55, 58]. Among 418 non-demented participants of the Religious Order Study (mean age 88.5±5.3 years), 35% showed macroscopic infarcts; those without these had microinfarcts (7.9%), arteriosclerosis (14.8%), both (5.7%), only 37.5% being free of CVLs [63]. In another study of 336 cognitively normal elderly adults, cerebral microinfarcts were seen in 33% and high-level microinfarcts in 10% [65], while among 100 non-demented aged subjects (mean age 81.2±5.4 years), CVLs including basal ganglia/deep white matter lacunes were seen in 73% and CAA in 39%; only 9% were free of CVLs [66]. There were no correlations between CVLs and AD related pathology in this latter cohort, whereas others reported an inverse relation between Braak neuritic stage and CVLs in autopsy-proven AD [67, 68]. The profile of AD and vascular changes becomes more complex with increased cognitive impairment in non-demented older and these changes are likely to constitute a major substrate for age-associated cognitive impairment, suggesting a need for rigorous investigation of both neurodegenerative and vascular risk factors in old age [61]. However, the interactions in the pathophysiology between vascular risk factors, CVD and AD pathology, while plausible, are still unresolved.

In contrast to AD, less is known about the impact of CVD in other common neurodegenerative diseases, i.e., dementia with Lewy bodies (DLB) and frontotemporal lobar degeneration (FTLD). Prevalence reports of CVD in DLB are scarce, but autopsy studies reported a frequency of 20.2-34.4% [69, 70], which does not differ significantly from controls [70]. In addition, an autopsy study indicated that more advanced Lewy body pathology is less likely to show severe CVD and therefore suggested that cognitive impairment in DLB appears to be independent from CVD [71]. With regards to the heterogeneous group of FTLD, data in relation to the prevalence and patho-mechanistic role of CVD is very limited and contradictory. One autopsy study reported a frequency of 5.2% for FTLD-tau and 17.3% for FTLD-TDP-43 [69]. Some data support a role of SVD in FTLD disease progression [72], while others could not confirm this [69]. Therefore, further studies are necessary to clarify the role of CVD in non-AD neurodegenerative diseases.

In conclusion, the co-occurrence of CVD and AD in the elderly is very frequent [73]. There is evidence suggesting that both lead in an additive as well as an independent fashion to cognitive dysfunction. The characteristic pattern of HPτ -related neurodegeneration (i.e., Braak NFT stages) in AD corresponds to a pattern of memory loss which spreading to other cognitive domains. By contrast, the neuropsychological profile associated with VaD shows considerable variation; e.g., executive dysfunction often equals or may exceed memory impairment in the SVD-subtype of VaD, but depending on location and severity of cerebrovascular lesions all possible types of cognitive impairment may ensue. We anticipate that the availability of comparable measures of AD and VaD pathology from *in vivo* neuroimaging studies in the future will replace dichotomous classifications of diseases with more sophisticated modeling. However, as of today, the best available models predict less than half of the variance in cognitive performance [49].

White matter hyperintensities

WML histologically encompass structural damage of the cerebral white matter as a result of white matter rarefaction [3] and are visualized as WMH on *pre*- and *post* mortem T2-weighted MRI, and have been associated with a wide range of cognitive deficits [74]. Interestingly, WMH are frequently seen in both demented and non-demented individuals, although WMH seen in AD are significantly more severe than the ones seen in so-called "normal ageing" [75-77]. The pathogenesis of WMH is generally thought to be associated with SVD as vessel wall alterations may lead to chronic hypoperfusion of the surrounding white matter [35]. Although WMH are currently assumed to reflect SVD, WMH on T2-weighted MRI are a visualization of white matter abnormalities and cannot determine the underlying pathogenesis. Previous studies have suggested a multifactorial aetiology of WMH [78-82] inclusive of SVD- related ischaemia, but also degenerative axonal loss secondary to cortical AD pathology, i.e., deposits of HPτ and Aβ. The exact pathological mechanism of degenerative axonal loss is still unclear, but it is suggested axonal death occurs simultaneous to grey matter atrophy, or via calpain mediated degradation, activated by AD pathology-related axonal transport dysfunction [83, 84]. Evidence from neuroimaging has shown region-specific white matter changes in AD patients, most frequently in the posterior deep white matter [75, 85, 86] and corpus callosum [75], which have been directly associated with AD-related cortical atrophy [85, 86].

HPτ has been implicated as a principle instigator of degenerative axonal loss in AD. An extensive quantitative neuropathological study revealed that the burden of cortical HPτ in the temporal and parietal lobes was a predictor of WMH severity in AD [87] corroborating previous studies reporting an association between higher Braak NFT stage and an increased WMH severity [77, 78, 88], and degenerative axonal loss in temporal [89] and parietal [84] white matter when in the proximity of high cortical HPτ pathology burden.

Furthermore, the combination of high CSF total-tau and higher parietal WMH volume was shown to be a predictor for the clinical conversion from mild cognitive impairment to AD [90], further supporting an association between the two pathologies. Although SVD-related ischaemic damage has long been assumed to be the main factor for the development of WMH (for review see [91]), neuropathological investigations of AD subjects with severe WMH usually revealed only minimal SVD pathology [84, 89, 92]. However, in cases with minimal neocortical HPτ pathology (Braak NFT stage 0-II) SVD was found to be associated with WMH [93] (Figure 2).

While theoretically both cortical HPτ pathology and SVD may lead to the development of WMH it appears that in neurodegenerative diseases, such as AD, WMH are likely to be primarily associated with cortical HPτ pathology. On the other hand, in non-demented and VaD cases, SVD seems to play a role in the development of WMH, which may relate to gliovascular abnormalities and blood-brain barrier (BBB) damage [94]. The clarification of the underlying pathogenesis of WMH and respective MRI characteristics is warranted to allow for clear interpretation of white matter neuroimaging and subsequent adequate management of patients.

Cerebral microbleeds

The term cerebral microbleeds describes the radiological phenomenon of small, well-demarcated, hypointense, round or ovoid lesions detected on T2*-weighted gradient-recalled echo (T2*-GRE) and susceptibility-weighted imaging (SWI) MRI sequences [10]. microbleeds create a “blooming” effect on T2*-GRE/SWI, but are generally not well seen on T1-weighted or T2-weighted sequences [10, 95]. Microbleeds have generated interest as a marker of the haemorrhagic consequences of SVD. Microbleeds are common in many different patient populations (healthy elderly, ischaemic stroke, intracerebral haemorrhage [96, 97], AD [98, 99] and VCI [100] 30)). Of note, microbleeds are more prevalent in patients with recurrent stroke than in those with first-ever stroke, and they tend to accumulate over time, indicating a relationship with progression and severity of cerebrovascular pathology [96]. Microbleeds generate increasingly common clinical dilemmas due to concern that they may be a marker of future intracerebral bleeding risk [101-106]. In a meta-analysis of 10 prospective studies including 3067 patients with ischaemic stroke or transient ischaemic attack, microbleeds presence was associated with a high risk of intracerebral haemorrhage (pooled odds ratio 8.53) raising questions regarding safety of antithrombotic drugs [107, 108]. Moreover, most available studies suggest that microbleeds are associated with impairment of cognitive function [109, 110], although whether they are directly and independently implicated - or simply reflect more severe SVD - remains uncertain.

Similar to other SVD markers, microbleeds appear to represent a potential link between stroke, brain ageing, dementia, and AD [99, 111], but have not yet resulted in high quality evidence-based recommendations for stroke and dementia clinical practice, nor emerged as a valid surrogate marker for clinical trials in SVD, e.g., intracerebral haemorrhage and VCI. This might be due to the significant gap between the clearly defined marker seen on MRI and their as-yet uncertain pathological basis and pathophysiological mechanisms [111-114]. It is consistently emphasized in the relevant literature that microbleeds are the MRI correlate of extravasation of red blood cells from arterioles and capillaries damaged by a primary haemorrhagic SVD process, and, hence, potentially strongly associated with haemorrhagic stroke risk. However, microbleeds are also associated with increased subsequent ischaemic stroke risk [115-118], highlighting that they are a marker of a CVD that is simultaneously ischaemic and haemorrhagic, a phenomenon sometimes termed mixed CVD [111, 119]. Nonetheless, histopathological correlation studies suggest that radiologically-defined microbleeds generally correlate with focal deposits of blood-breakdown products, predominantly haemosiderin-iron [112, 120]. MRI-histopathological correlation, has been underutilized [121, 122], with a total of fewer than 70 microbleeds been analysed in just a small sample of patients [112-114], often detected using relatively insensitive T2*-GRE sequences at 1.5 T [120]. Technical challenges involved in correlating MRI with histopathology for such small lesions with a widespread distribution in the brain probably account for the small number of brains with microbleeds that have been analysed. Notwithstanding these limitations, when systematic neuropathologic examination of SWI-visualized microbleeds is undertaken, the underlying pathologic substrates are actually rather variable, including not only focal accumulations of blood-breakdown products, but also (albeit much less commonly) microaneurysms, small lacunes, vessel wall dissections or (pseudo-) microaneurysms [114, 120, 123, 124].

Although most microbleeds pathological correlation studies emphasize blood leakage from nearby damaged small vessels into the brain parenchyma as a mechanism, it must not be assumed that a primary haemorrhagic process fundamentally produces all microbleeds or that the most severely affected vessels are the culprits. Alternative non-haemorrhagic mechanisms for microbleeds, particularly if no tissue damage surrounds the vessel and haemosiderin is limited to perivascular space, include ischaemia-mediated iron store release by oligodendrocytes [125], phagocytosis of red blood cell microemboli into the perivascular space (termed angiophagy) [123, 126], or even haemorrhagic transformation of small “microinfarcts” [127] (Figure 3).

It is widely accepted that, by analogy with spontaneous intracerebral haemorrhage, the pathological processes underlying microbleeds differ according to their location in the brain, with CAA being the most notable correlate of exclusively lobar microbleeds (most often in the occipital and posterior temporo-parietal

regions), while 'hypertensive arteriopathy' (including a spectrum of neuropathological processes affecting deep perforating vessels such as AS, lipoohyalinosis etc.) is strongly associated with predominantly but not exclusively deep microbleeds. The majority of data to date support this hypothesis, but much of the evidence is indirect and largely based on clinical and imaging studies [10, 114, 128-132], rather than extensive direct morphological-pathological analyses [133]. A recent neuropathological study found no direct topographical association between CAA presence or severity and microbleeds (defined only pathologically as haemosiderin-laden macrophages in any brain region) [134]. Whether these microscopic lesions have the same biological significance and underlying mechanisms as radiologically defined microbleeds is not clear [122]. Further exploring the neuropathological basis of microbleeds will be a key step in clarifying their mechanisms and nature, and along with well-designed observational clinical studies will help microbleeds to become useful for clinical management decisions [135]. Until then, the main question, i.e. whether a radiologically-defined microbleed really always is a true microbleed or may also represent haemosiderin deposits which in turn may or may not stem from a microbleeding event, remains unanswered.

Additional novel approaches to complement and enhance current *post mortem* assessment of cerebral human tissue

With regards to cerebrovascular lesions, novel applications of neuroimaging and biochemical methods, as well as additional investigation of neuroinflammation, have been suggested for assessment of human *post mortem* brains. Although these methods are beyond the scope of basic routine diagnostic procedures, the addition of such novel techniques may help to further elucidate the impact of CVD on cognitive performance.

Post mortem neuroimaging

Post mortem MRI provides a technique to complement research, and routine, neuropathological investigations, providing visualization of cerebral lesions for radiological assessment or a precise location for histological examination. Direct comparison studies have found that gross MRI lesions were almost identical between human *in-vivo* and *post mortem* MRI imaging [136], with limited effects on MRI characteristics due to the fixation process [137, 138]. A variety of *post mortem* MRI approaches have been implemented including scanning of fixed whole brains or hemispheres [77, 136, 137, 139-143], coronal brain slices [144, 145], un-fixed whole brains [136] and brains in situ [146].

Frequently, *post mortem* MRI is used for the detection and assessment of WMH and a recent study [142] investigated the reliability of *post mortem* MRI for assessment of WMH of the deep white matter; 40 *post mortem* right fixed brain hemispheres underwent a 4.7 T MRI scan with WMH in the deep white matter were

rated according to the Age Related White Matter Change Scale (ARWMC) [147] and compared to scores from a thorough histological assessment (based on approximately 1200 sections). The study revealed no significant differences between the *post mortem* MRI WMH scores and histological assessments, regardless of the severity of the deep white matter changes, demonstrating that *post mortem* MRI is a reliable measure of WMH that can be utilized to complement neuropathological assessment of white matter changes. Of note, routine histological assessment based on 5 histological sections per brain, failed to reliably reflect thorough histological assessment.

Cortical microinfarcts (CMI) are another common lesion found in ageing and dementia, and are considered the "invisible lesions" in clinical–radiological correlation studies [148], visible only upon microscopic examination. Developments in high-resolution 7.0 T MRI have allowed for the detection of CMI *in-vivo* [43]. This approach was utilized and established for the *post-mortem* detection of several types of CMI by De Reuck and colleagues [45]; fixed coronal slices from 175 demented and non-demented brains underwent a 7.0 T MRI and mean CMI and cerebral CMI loads were determined and compared to the histological examination revealing no statistical differences between the two assessments.

Post mortem MRI has also proved a valuable tool in investigating the pathomechanisms of ischaemic stroke in the human brain and this is of major potential importance as many therapeutic interventions that have been proven successful in animal stroke models, have not yet been verified in human clinical trials (excluding thrombolysis and hypothermia). Developments in autoradiography of intact human brain sections have allowed for the visualization of the ischaemic core by creating a 'potassium map'; a method which identifies the ischaemic core by utilizing the disruption of ion homeostasis and subsequent efflux of water. This method allows for the essential targeted tissue sampling of the ischaemic core to facilitate quantitative measurements of tissue components. The method for human brain sections, as described by Csiba and colleagues [149], is reliant upon *post mortem* MRI (T1 and T2 weighted) to localize the ischaemic lesions and serve as a gold standard comparison to the 'potassium map'. Of note, *in vivo* MRI imaging is not appropriate due to the possibility of new focal ischaemic lesions developing. Following *post mortem* MRI, the brain is frozen and the region of interest, i.e., brain infarct with the perifocal brain tissue, is cryosectioned using a heavy-duty microtome (LKB 2250 PMV Cryo-microtome) eventually the entire hemisphere that can cut and examined. On the cryosections the necrotic core, penumbra and perilesional brain can be identified using the 'potassium map' method [150], with specific samples removed via a micropunch technique [151] allowing for subsequent analysis of water content, proteomics and genetics. Although this combined methodology of *post mortem* MRI and potassium mapping is beyond the scope of the routine diagnostic work-up, it is unparalleled in providing the targeted tissue sampling for the *post mortem* examination of an

ischaemic brain in the research setting.

Biochemical assessment

Whilst clinical, neuroimaging and pathological assessment remain the main approaches for the assessment of vascular lesions and their association with cognitive impairment and other neurological disturbances, *post mortem* biochemistry provides additional insights into vascular function [152]. Biochemical assays enable us to measure and investigate the mechanisms of vascular dysfunction, including the activity and level of enzymes and proteins that mediate changes in vascular calibre, permeability and adhesion, cell migration, vascular maintenance and regeneration, as well as structural proteins the measurement of which provides quantitative data on a wide range of vascular and parenchymal cells and extracellular constituents.

Advantages of including biochemical measurements (in addition to more conventional morphological assessments) include the fact that they are more sensitive for the detection of hypoperfusion, facilitate more representative sampling (e.g. up to 0.5 ml of tissue in a single homogenate c/w about 5µl of tissue in a paraffin section) and yield objective continuous data rather than subjective ordinal scores. Biochemical approaches were recently used to gain some understanding of the pathogenesis of cerebral hypoperfusion in VaD, AD and DLB. Measurement of the levels of myelin proteins with long half-lives but differential susceptibility to hypoperfusion confirmed significant reduction in perfusion of the cerebral cortex and white matter in VaD [39, 153]. This was evidenced by a decline in the ratio of myelin-associated glycoprotein (MAG) to proteolipid protein 1 (PLP1). Whereas PLP1 is distributed throughout the myelin sheath, MAG is located in the adaxonal loop of myelin, the first part of the myelin sheath to degenerate when blood supply is inadequate to meet the energy requirements of the oligodendrocyte (Figure 4). Biochemical analysis confirmed the significant decline in perfusion of the cerebral cortex in AD as well as VaD [154]. The reduction of MAG:PLP1 ratio was demonstrable in early AD (Braak NFT stages III and IV) in the precuneus (the first region of cortex to affected by a decline in blood flow in AD) indicates that the perfusion is inadequate to meet metabolic demand rather than that the hypoperfusion is simply a reflection of reduced metabolic activity [155]. The hypoperfusion in AD could not be attributed to SVD or CAA, with which there was no significant association. However, the severity of hypoperfusion was associated with a marked increase in the concentration of the vasoconstrictor endothelin-1 (EDN1) in the cerebral cortex in AD. Correlation between the level of EDN1 and that of Aβ42 was also demonstrated, suggesting that a driver for the production of the EDN1 is the accumulation of this peptide, which up regulates neuronal production of EDN1 by endothelin-converting enzyme-2 [156] (in contrast, the level of EDN1 did not correlate with that of Aβ40, which up regulates endothelial production of EDN1 by endothelin-converting enzyme-1 [157, 158]).

In the cerebral white matter, the main abnormality associated with hypoperfusion in both VaD and AD was non-amyloid SVD [39]. The concentration of EDN-1 in the white matter was reduced in AD, as was that of another vasoconstrictor, angiotensin II, and the activity of angiotensin-converting enzyme, the enzyme responsible for angiotensin II production [155]; these are likely to be adaptive responses to reduced perfusion. However, perfusion of the white matter (as measured by the MAG:PLP1 ratio) has been shown to fall with increasing EDN-1 in the overlying cortex, suggesting that vasoconstriction of perforating arterioles within the cortex probably contributes to hypoperfusion of the underlying white matter in AD.

Additionally, the concentration of von Willebrand factor (VWF) in brain tissue is directly related to the density of microvessels [154, 159]. Measurement of VWF has several advantages over quantitative immunohistochemical methods of assessing microvessel density: the sample size can be much larger (a 0.5 ml homogenate contains ~10^6-fold greater volume of tissue than a typical paraffin section) and the same homogenate can be used for measurement of a wide range of related molecules, allowing direct comparison between microvessel density and perfusion, vascular function, and molecules responsible for regulation of vascular growth, tone and permeability. This approach was used to assess possible causes of occipital hypoperfusion in DLB, and demonstrated significant reduction in the level of VWF in the occipital cortex (a region known to be hypoperfused in DLB) but not midfrontal cortex or thalamus [159]. Furthermore, reduction of VWF correlated with loss of MAG (a marker of hypoperfusion, as noted above), as well as reduction in the level of vascular endothelial growth factor (VEGF), which is needed for maintenance of the vasculature. Finally, reduction in VEGF was revealed to related to the level of α-synuclein, not only in the *post mortem* human brain tissue but also in neuronal cell lines engineered to over-express wild-type α-synuclein, suggesting that α-synuclein may down regulate production of VEGF, affecting maintenance of the microvasculature and of cerebral perfusion.

These few examples illustrate the potential of *post mortem* biochemical analysis of brain tissue as a means to measure vascular function and to investigate the pathogenesis of vascular dysfunction.

Neuroinflammation - a contributor to vascular dementia?

Aside from the hallmark pathological lesions, there is evidence to suggest a role for immunological and inflammatory mechanisms in the pathophysiology of VaD/VCI. Neuroinflammation encompasses local endothelial activation, leading to the extravasation of fluid (and sometimes, cells) via a dysfunctional BBB resulting in oedema and tissue damage in the surrounding parenchyma, eventually leading to the activation of perivascular macrophages, microglia, and other glial subtypes [160-162] (Figure 5 A, B)

Clinical studies in patients with symptomatic SVD [163, 164] or WMH [165-167], found elevated circulating biomarkers of endothelial activation i.e., ICAM1, soluble thrombomodulin, interleukin-6, PAI-1. This suggested that endothelial activation, and a possible inflammatory process, might contribute to SVD, and cognitive decline. A neuropathological study by Giwa and colleagues assessed endothelial activation in small perforating arteries in cases with moderate-severe SVD, and with minimal AD pathology (Braak NFT stage 0-II, and insufficient neuritic plaque pathology to meet CERAD criteria for AD. They found that endothelia were rarely immunoreactive for ICAM1 or IL-6, however, luminal thrombomodulin (depletion of which is a hallmark of activated endothelium) was more pronounced, especially individual vessels with severe high sclerotic index [168] (Figure 5 C). The study concluded that local endothelial activation is not a feature of the arteriolosclerosis form of SVD, in agreement with evidence from a previous study of brain lysates demonstrating attenuation of inflammatory mediators (MCP-1 and IL-6) in individuals with VaD and mixed dementia, relative to aged control subjects [169]. While BBB dysfunction is often claimed to be part of SVD pathology, neuropathology studies show no conclusive association of BBB markers (fibrinogen, IgG, albumin; Figure 5 D) with SVD. Some neuropathology reports found a positive association between SVD severity and extravascular plasma proteins [170, 171] while others did not [141, 172, 173]. In subcortical white matter, fibrinogen labelling was associated with clinical dementia diagnosis in an AD-free cohort where dementia is likely to be primarily VaD [173]. Observationally, little evidence of leukocyte infiltration is associated with SVD. Microglia have been shown to be significantly higher in number in brains of persons with VaD and widespread WMH [79, 174, 175]. Activated microglia (CD68+) are strongly associated with WML [79, 145] (Figure 5 E and F)

Elucidation of the role of neuroimflammation in the pathogenesis and pathophysiology of SVD will enable the evaluation of immunotherapies as potential therapeutic options for prevention or treatment of VCI/VaD.

Conclusion and outlook

It becomes increasingly clear that standardised neuropathological criteria for the assessment of CVD in human *post mortem* brains are needed [176]. In order to establish such criteria, Brains for Dementia Research initiated a UK multi-centre collaborative study to formulate evidenced-based Vascular Cognitive Impairment Neuropathology Guidelines (VCING) for *post mortem* assessment of CVD of relevance to VCI. Nine neuropathologists undertook a Delphi method series of surveys to agree on a neuropathological sampling protocol and scoring criteria that included assessment of 14 vessel and parenchymal pathologies in 13 brain regions. To validate VCING the neuropathologists performed blinded assessment of 114 brains

from people with little or no AD (Braak tangle stage ≤III) or Lewy body pathology. Inter-rater reliability analyses showed VCING to be reproducible, with almost perfect agreement amongst neuropathologists (AC2 coefficient >0.8 [177]) for most scoring, apart from that of AS and microinfarcts, which was more variable (0.4 to ≤0.8). Regression analyses identified seven pathologies as individual predictors of VCI. Modeling identified the best combination of area-pathologies (area under ROC curve 72%) predicting probabilities of VCI from 20%-90% depending on the combination of pathologies [178].

In addition to the refinement of routine neuropathological scoring criteria, complementary methods such as *post mortem* MRI and biochemical assessment are promising tools to investigate CVD. Not only should these be helpful to better understand the pathophysiology of VCI/VaD but also clarify the pathophysiological processes that ultimately lead to characteristic findings of *in vivo* imaging. The latter seems a timely need, since current assumptions regarding the "causes" for WMH and cerebral microbleeds may not be accurate in all cases and, hence, negatively impact on the diagnostic accuracy of respective clinical diagnoses.

Abbreviations

Aβ, amyloid-beta; AD, Alzheimer's disease; ARWMC, age related white matter change; AS, atherosclerosis; BBB, blood-brain barrier; CAA, cerebral amyloid angiopathy; CMI, cortical microinfarcts; CVD, cerebrovascular disease; DLB, dementia with Lewy bodies; EDN1, endothelin 1; FTLD, frontotemporal lobar degeneration; HP τ , hyperphosphorylated tau; LBD, Lewy bodies disease; MAG, myelin-associated glycoprotein; MRI, magnetic resonance imaging; NFT, neurofibrillary tangle; PLP1, proteolipid protein 1; SVD, cerebral small vessel disease; SVD-AS, small vessel disease atherosclerosis; SWI, susceptibility-weighted imaging; VaD, vascular dementia; VCI, vascular cognitive impairment; VCING, vascular cognitive impairment neuropathological guidelines; VWF, von Willebrand factor; WMH, white matter hyperintensities; WML, white matter lesions

Competing interests

The authors declare that they have no competing interests.

Funding

KE.M is currently supported by the by the Alzheimer's Society. LT.G was funded by institutional NIH grants (P50AG023501, P01AG019724, and R01 AG040311). T.H has received support from the Hungarian Brain

Research Program (KTIA_13_NAP-A-II/7). Cerebral tissue for some studies in this consensus was provided by the Newcastle Brain Tissue Resource, which is funded in part by a grant from the UK Medical Research Council (grant number G0400074) and by Brains for Dementia research, a joint venture between Alzheimer's Society and Alzheimer's Research UK.

Author's contribution

Review concept and design was conceived by J.A. Manuscript contributions by KE.M, A.C, J.DR, LT.G, AH.H, T.H, K.J, S.L, O.S. Final manuscript drafted by KE.M and J.A. Critical revisions and general consensus by I.A, LT.G, T.H, P.I, K.J, J.G, RN.K, GG.K, E.K, S.L, M.P, R.T, DR.T, D.W, SB.W and J.A. Figures 1 and 2 were prepared by KE.M, figure 4 was prepared by S.L, and figure 5 was prepared by AH.H.

Acknowledgements

We are grateful to the individuals and their families who kindly donated their brains for research.

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Figure legends

Figure 1

Schematic diagram illustrating the three most commonly observed cerebrovascular diseases and their resulting cerebrovascular lesions that may lead to specific types of vascular dementia.

Figure 2

A series of images for three separate cases indicating normal appearing white matter and the similarity of white matter changes with differing pathogenesis in the deep white matter of the parietal lobe (Brodman area 39/40), as seen on both T2-weighted MRI and histologically. Images A-Aiv corresponds to a normal aged control brain with no obvious white matter changes or small vessel disease (SVD), and no Alzheimer's

disease (AD) -related pathology. A, *post mortem* T2-weighted MRI of normal appearing white matter; Ai-Aii, corresponding histological magnified image of normal appearing white matter and a normal white matter artery (Aii); Aiv, overlying cortex with no hyperphosphorylated tau (HPτ) pathology. Images B-Biv corresponds to a normally aged case that exhibited severe white matter hyper intensities/lesions with SVD but no AD pathology. B, *post mortem* T2-weighted MR image indicating confluent white matter hyper intensity (WMH); Bi, corresponding histological magnified image of white matter lesion indicated by widespread pallor of the central white matter with typical sparing of the subcortical U-fibres (arrow); Bii, higher magnification of white matter lesion exhibiting severe rarefaction, i.e., myelin and axonal loss; Biii, white matter arterioles from white matter lesion area exhibiting arteriolosclerosis with hyalinisation (arrows) of vessel walls; Biv, overlying cortex with no HPτ pathology. In this case one may speculate SVD-related hypoperfusion was the primary cause of white matter changes. Images C-Civ corresponds to an AD brain exhibiting severe white matter hyperintensities/lesions and no obvious SVD. C, *post mortem* T2-weighted MR image indicating confluent white WMH; Ci, white matter lesion with severe white matter pallor; Cii, magnified image of severe white matter rarefaction; Ciii, white matter arteriole with enlarged perivascular space but no small vessel disease-related fibrosis or hyalinisation; Civ, overlying parietal cortex exhibiting severe HPτ pathology. In this case one may speculate white matter changes were the result of degenerative myelin and axonal loss as a result of grey matter atrophy in the overlying cortex or via protease mediated degradation, activated by AD pathology-related axonal transport dysfunction. MRI scans captured in sagittal plane. Microphotoimages captured from serial sections. Histological stain Luxol fast blue was used for images Ai-ii, Bi-ii, and Ci-ii; H&E stain was used for Aiii, Biii and Ciii. Immunohistochemistry, AT8 antibody for Aiv, Biv, Civ. *Scale bars* represent 1000μm in images A, B and C and 20μm in images Ai-iii, Bi-iii, Ci-iii.

Figure 3

MRI and histological sections of cerebral tissue exhibiting microhaemorrhages. A, radiological characteristics of microhaemorrhages inclusive of small, well-demarcated hypointense ovoid lesions (arrow). B-Ci, images from an 81-year old male with dementia and severe CAA on pathology, B, p*ost-mortem* 7T MRI image of hypointense ovoid lesion (arrow). C, magnified image of cortical microhaemorrhage. Ci, increased magnified image of cortical microhaemorrhage; brown deposits are haemosiderin (arrow) and yellow deposit is haematoidin (arrow head) indicating the microhaemorrhage is subacute. Histological stain H&E used on images C and Ci. Scale bars represent 1000μm in image C, and 100μm in image Ci. Images prepared by Dr S. van Veluw.

Figure 4

Schematic illustration of the distribution of MAG (pink dots) and PLP1 (green dots) in the myelin sheath. When the supply of oxygen and glucose is insufficient to meet the metabolic needs of the oligodendrocyte, as occurs in hypoperfusion, the first part of the cell to degenerate is the adaxonal loop of myelin - the part of the oligodendrocyte that is furthest away from the cell body (so-called dying back oligodendrogliopathy). Because MAG is restricted to the adaxonal loop of myelin whereas PLP1 is widely distributed throughout the myelin sheath, hypoperfusion leads to greater loss of MAG than PLP1. In contrast, degeneration of nerve fibres causes loss of both MAG and PLP1. The severity of *ante mortem,* hypoperfusion can be assessed by measuring the MAG:PLP1 ratio. Illustration from [179] with permission from Prof. S. Love.

Figure 5

Neuro-inflammatory markers in donated human brain tissue, from older people. A, Immunohistochemical labelling for the pan-selective microglial marker Iba-1. B, activated microglia in a phagocytic state, with amoeboid morphology, immunoreactive for lysosomal marker CD68 (clone PGM1). C, immunoreactivity for endothelial marker thrombomodulin (TM) in a small penetrating artery of the anterior putamen. D, immunoreactivity for the large plasma protein fibrinogen (FGEN), in deep subcortical white matter. Perivascular cells with astrocytic morphology show cellular labeling (arrows). E, a localized cluster of activated microglia (CD68+ (PGM1)), indicating a focal white matter lesion within deep subcortical white matter. F, (magnified image of image E) exhibiting a small arterial vessel. Haematoxylin counterstain was used in A-F. Scale bars represent 20μm in images A, B and C; 100μm in image E, and 50μm in images D and F.

Figure 1

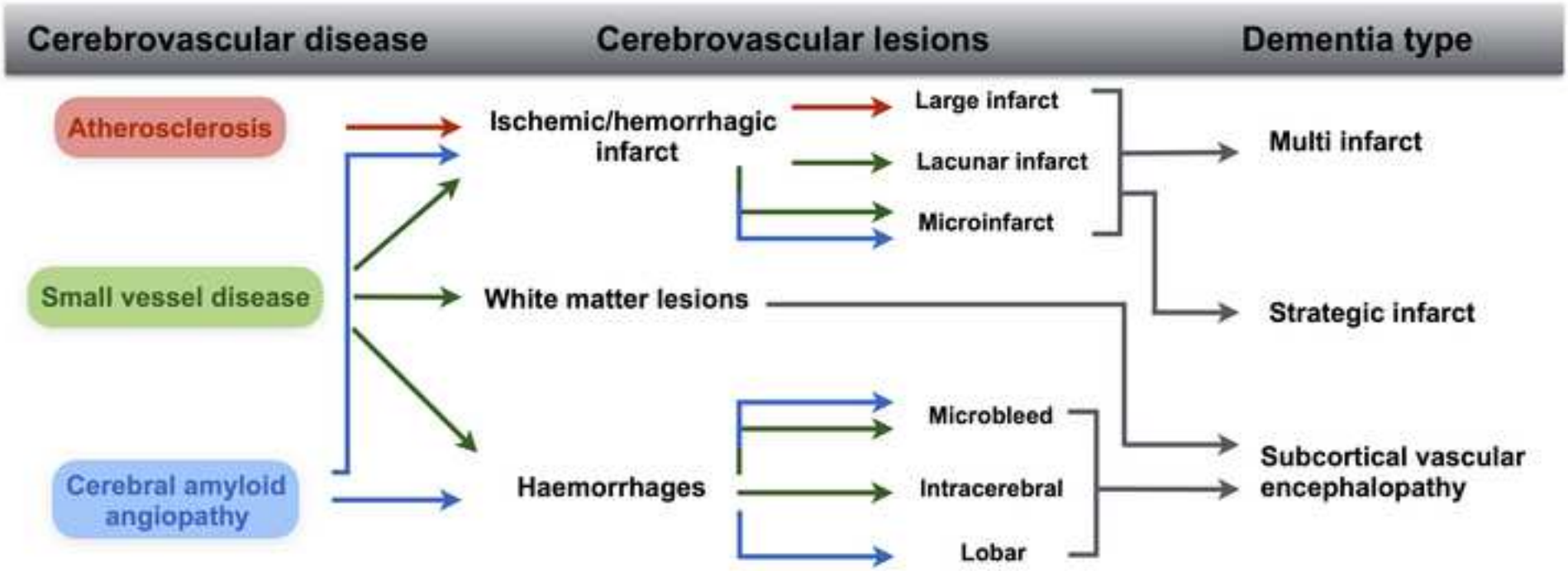

Figure 2

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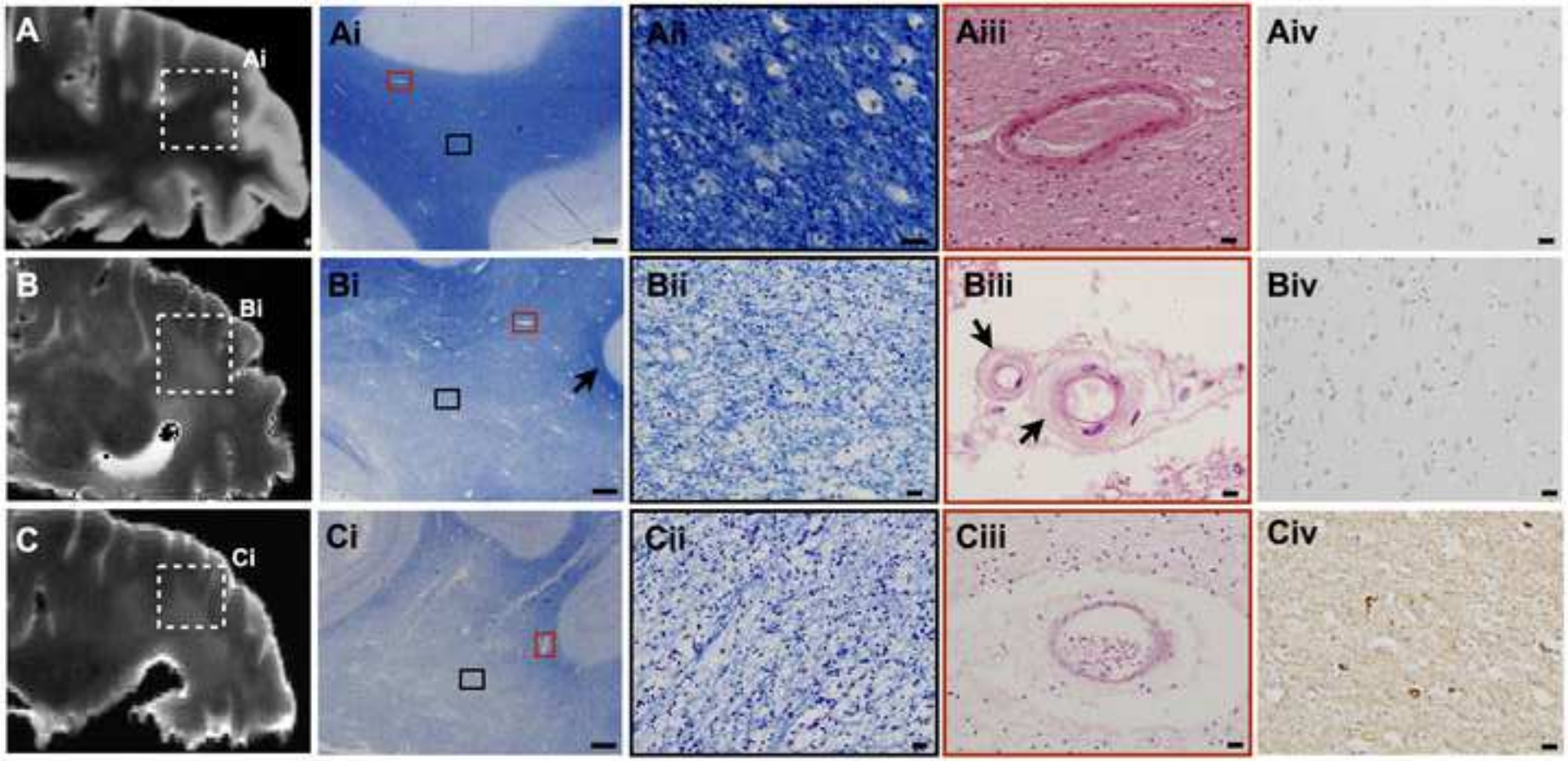

Figure 3

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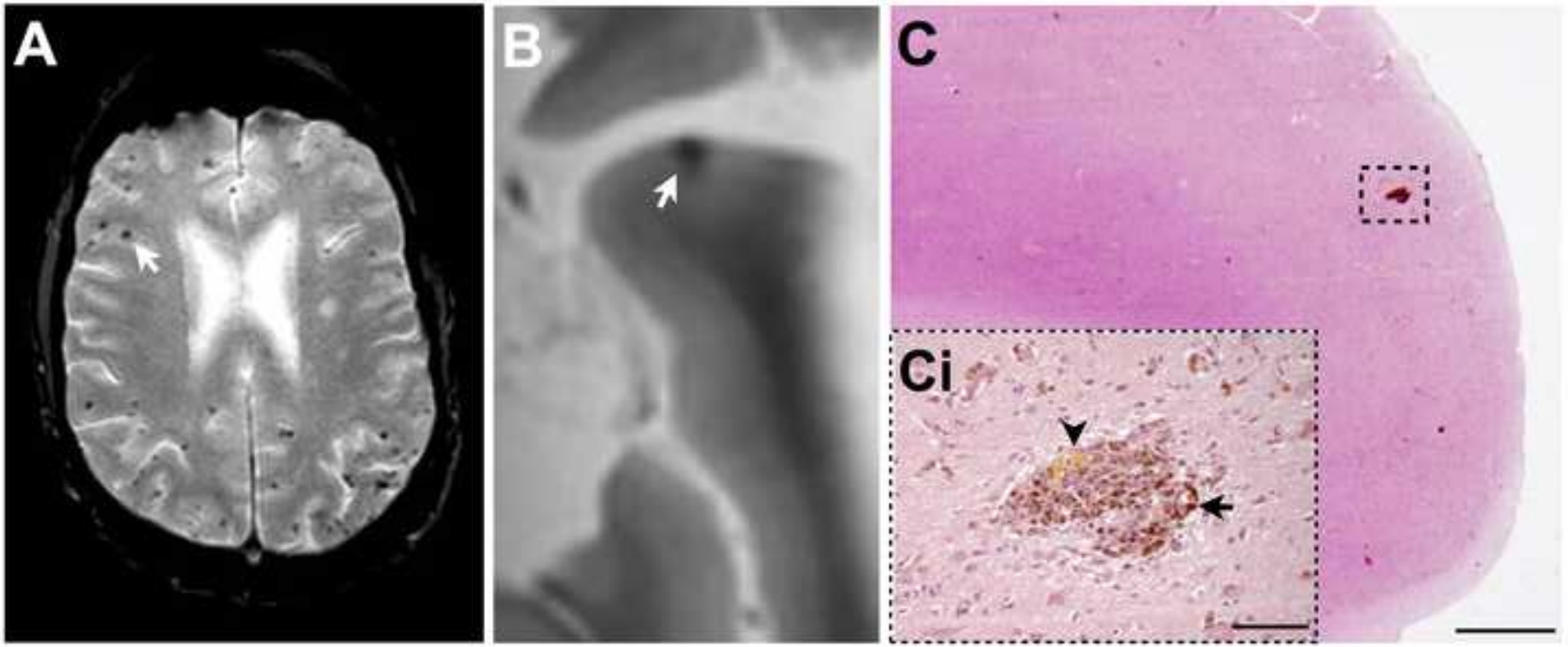

Figure 4

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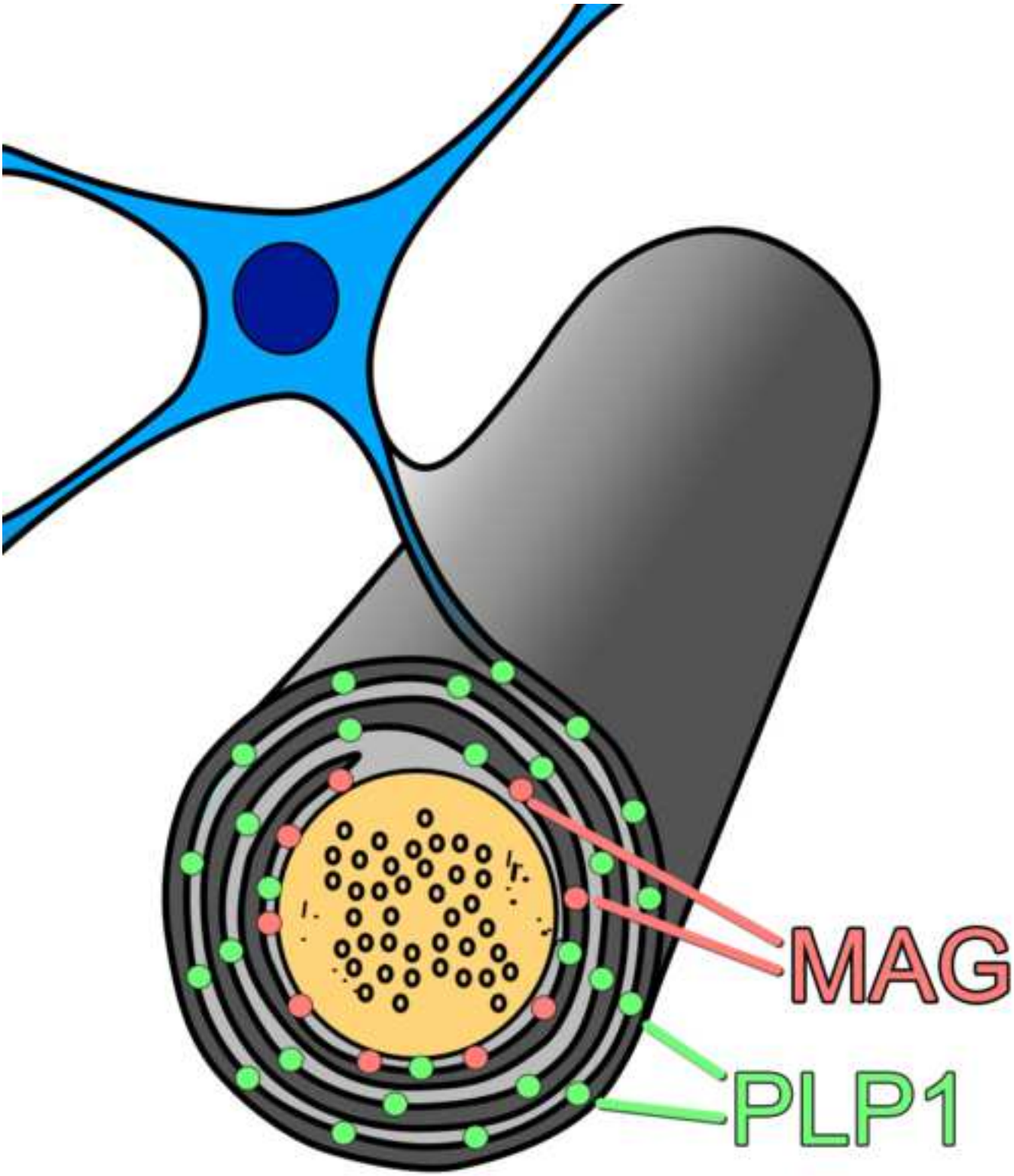

Figure 5

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