Advanced Neuroimaging to Unravel Mechanisms of Cerebral Small Vessel Diseases

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Cerebral small vessel disease (cSVD) is the umbrella term used to describe pathologies of the vascular structures (small arteries, arterioles, capillaries, small veins, and venules) that are located in the brain parenchyma or the subarachnoid space.[1] Despite the fact that some pathological processes can damage both large and small vessels, there is ample evidence suggesting that these conditions have distinct mechanisms and clinical consequences. Even common risk factors, such as hypertension, have discernible effects on the cerebral large vessels (intracranial atherosclerosis) and small vessels (white matter disease, lacunar infarcts as well as intracerebral hemorrhages [ICHs] and microbleeds).[2] Research into the causes and consequences of cSVD have blossomed over the past 2 decades, improving our understanding of the relevance of these microangiopathies as causes of ischemic strokes, ICHs as well as contributors to cognitive impairment, gait disorders and behavioral changes in older adults.[3,4] Appropriate diagnosis of cSVDs is clinically relevant to select appropriate stroke prevention methods, and research into their mechanisms is key to decrease the burden of age-related dementia and motor impairments, major causes of disability and death.[5–7]

Until recently, the only way to estimate microstructural and molecular alterations in the human brains was to perform detailed histopathologic studies. Such studies are possible only in the context of a postmortem examination, at the tail end of the pathological evolution. The role of histopathologic evaluation is, therefore, limited in identifying the initial changes in the brain and their progression, processes that might take decades before strokes and microstructural changes culminate into motor and cognitive impairments. Understanding the early phases of such progression is key to stopping the pathological cascade before severe damage occurs. Understanding changes in cerebral vessel function and their pathological consequences constitute another major target in cSVD research.

Early physiological studies of the vessels in humans included mainly transcranial doppler based approaches that are typically limited to large intracranial vessels. Contrast-based perfusion studies depict hemodynamics at tissue level, but they had relatively low resolution and lacked the capacity to show dynamic changes in vessel function. Most of the research in the field of cerebrovascular physiology came from animal studies that do not always translate into successful diagnostic and therapeutic efforts in humans. For all these reasons, developing neuroimaging methods that can detect microstructural, molecular, and physiological alterations in living humans has been an important goal in cSVD research.[6,7]

The current article will review the contributions of advanced neuroimaging in studies evaluating the mechanisms of common sporadic cSVDs in living humans. The focus will be on studies that used cohorts of patients with relatively severe cerebral microangiopathies, such as primary ICH, where a distinction of the cSVD cause can be made. The aim is to discuss the novel imaging techniques and their applications to cSVD research and showcase results that currently guide our understanding of brain microangiopathies. We will specifically review advanced structural imaging, molecular neuroimaging, novel physiological imaging methods and their contributions to the cSVD field.

Advances in Structural Neuroimaging of Cerebral Microangiopathies

Types of cSVD

Multiple lines of evidence show that cSVDs result in both ischemic and hemorrhagic brain lesions. The 2 most common causes are hypertensive arteriolosclerosis and cerebral amyloid angiopathy (CAA). Hypertension is an exceedingly prevalent condition found in 63.1% of adults over 60 years of age in the United States, and hypertensive cSVD (HTN-cSVD) is the most common type of cSVD.[8,9] CAA refers to the accumulation of Aβ (amyloid-β peptides) in the walls of the leptomeningeal and cortical vessels.[10] Lacunar infarcts, white matter hyperintensities (WMH), ICH, and cerebral microbleeds (CMB) are tissue lesions detectable with brain imaging that are manifestations of cSVDs.[4] Neuroimaging studies have shown that topographical distribution of these lesions correlates with the type of the underlying microangiopathy. Recent work using both standard and advanced structural magnetic

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resonance imaging (MRI) also added new lesion types to the list of the classically accepted cSVD-related pathologies.

ICH and CMBs in deep-brain regions (basal ganglia, thalamus, and pons) are associated with severe HTN-cSVD, whereas ICH/MBs limited to superficial/lobar brain regions and cortical superficial siderosis are mainly seen in patients with CAA.[3] The presence and distribution of these hemorrhagic lesions still constitute the most important markers in the diagnosis and etiologic classification of cSVD in living individuals. Besides its diagnostic relevance, cortical superficial siderosis is also a very important marker of ICH recurrence risk in patients with CAA.[11,12] Use of susceptibility-weighted imaging MR at ultrahigh magnetic field strength (7 Tesla) answered an important question in CAA research, demonstrating that CAA-related microbleeds exclusively occur within the cortex (Figure 1).[13] Furthermore, the use of 7T susceptibility-weighted imaging MR revealed high incidence of novel hemorrhagic markers in hereditary cerebral hemorrhage with amyloidosis-Dutch type, namely intragyral hemorrhage (47% versus 0% in controls, $P<0.001$), and a high incidence of striped cortex sign (40% versus 0% in controls, $P<0.005$).[14] More widespread use of 7T susceptibility-weighted imaging in research is likely to result into better understanding of hemorrhagic lesions seen within the context of cSVDs.

Ischemic Markers of cSVD

White matter disease, also called leukoaraiosis, is defined as hypodensities on computerized tomography and WMH on T2/fluid attenuated inversion recovery MRIs in subcortical and periventricular regions that do not show cavitation or other characteristics of a completed infarction.[15] Multiple lines of evidence established leukoaraiosis as an important marker of cSVD. WMH volume was found to be significantly higher in patients with CAA and HTN-cSVD when compared with age-similar healthy adults as well as patients with mild cognitive impairment and Alzheimer disease.[16–18] WMH volume also correlated strongly with markers of severity of cSVD, both cross sectionally and longitudinally.[19,20] There has been remarkable interest in identifying WMH patterns that could distinguish different types of cSVD and even cSVD from other pathologies such as Alzheimer disease. Studies that used simple voxel-based comparisons failed to identify significant differences in WMH distribution probably because of the fact that the bulk of WMH is periventricular in all patients, therefore, no clear patterns emerged across different diagnostic categories after adjusting for age and the volume of leukoaraiosis.[17,18] A recent study that used visually identified WMH patterns showed differences between patients with CAA and HTN-cSVD. The presence of peribasal ganglia linear WMH was more commonly found in HTN-cSVD when compared with CAA (19% versus 7.8%; $P=0.001$) whereas having 10 or more small circular WMHs (subcortical spots) outside of periventricular locations was associated with the diagnosis of CAA (29.8% versus 16.8%; $P=0.004$).[21] One retrospective study that evaluated the center of WMH over the anteroposterior axis using computer-assisted segmentations suggested a more posterior distribution in patients with pathological evidence of CAA but no ICH.[22] More recent studies use deep learning to create segmentation algorithms and perform voxel-based spectral clustering analysis on aligned WMH maps to group image voxels into clusters, maximizing within-group and minimizing between-group similarities.[23] These studies confirm the previous findings of a deep WMH distribution in HTN-cSVD versus more peripheral and posterior WMH patterns in CAA.[23,24]

Classical lacunar infarcts are found distal to occlusive lesions of small perforating arteries mostly in basal ganglia, internal capsule, thalami, and pons—regions known to be supplied by deep branching/perforating vessels. These lesions are caused by distinct vessel pathologies that are categorized based on the size of the vessel: arteriosclerosis/atherosclerosis, arteriolosclerosis, and lipohyalinosis, collectively categorized as HTN-cSVD. There has been growing interest in lacunar infarcts in primary cSVD-related ICH as it is possible to identify the predominant microangiopathy in these patients during life. Lacunes were found in 25% of primary ICH patients. Lacunes in deep-brain locations were more commonly seen in patients with hypertensive deep ICH (15.2%) when compared to patients with CAA (2.1%, $P<0.001$). Interestingly, radiological lacunes in more superficial subcortical locations (lobar lacunes) were more common in CAA-ICH (20.4%) than HTN-ICH (5.7%, $P<0.001$; Figure 2).[25] Another study also found more common presence of lobar lacunes in CAA-ICH than in HTN-ICH (29.2% versus 11.6%, $P=0.036$), as

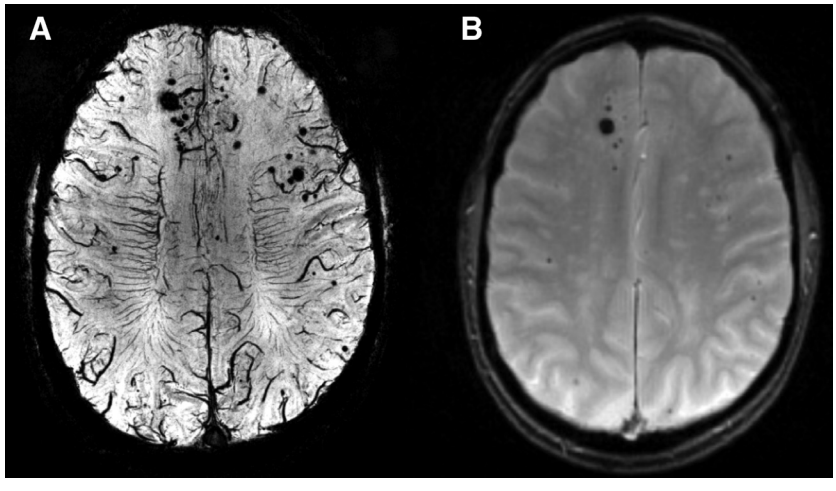

Figure 1. Impact of scanner/parameters on microbleed detection. Optimized susceptibility-weighted imaging obtained in Ultra-High Field (7T) magnetic resonance imaging (MRI) scanner (**A**) shows a much higher number of cortical microbleeds when compared with clinical-grade MRI (**B**) obtained on the same day in a patient with cerebral amyloid angiopathy.

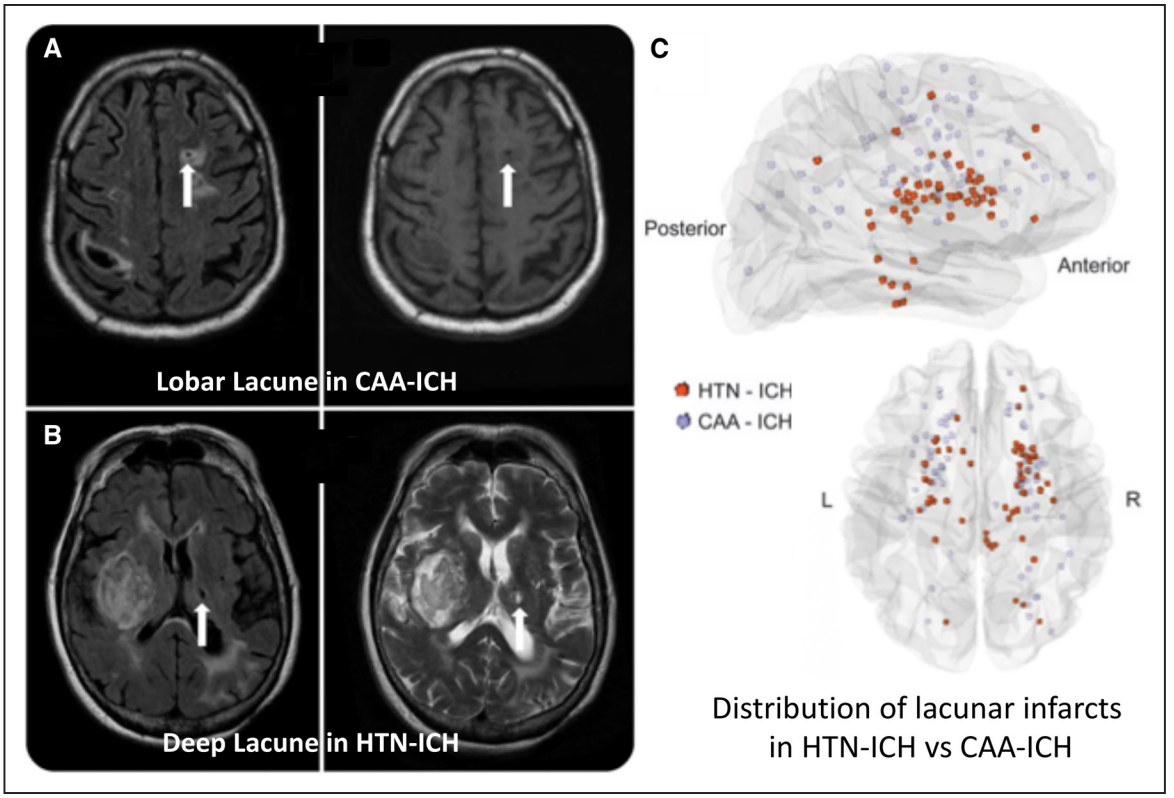

Figure 2. Distribution of lacunes in cerebral microangiopathies. Examples of lobar lacune (centrum semiovale) in cerebral amyloid angiopathy (CAA) (**A**), deep lacune in hypertensive deep intracerebral hemorrhage (HTN-ICH) (**B**) and topographical distribution maps of lacunes in these brain microangiopathies (**C**). Reproduced from Pasi et al[25] with permission. Copyright ©2017, Wolters Kluwer Health, Inc.

well as a significant correlation between lobar lacune counts and brain amyloid load (r=0.40, P=0.02).[26] These data establish radiological lacunes in lobar (nondeep) locations to be associated with CAA, a finding that might have both diagnostic and therapeutic implications.

Cerebral microinfarcts have classically been a histopathologic construct representing microscopic lesions with a mean diameter between 0.2 and 1.0 mm, found at autopsy in 16% to 46% of unselected elderly people dying of all causes.[27] A study that identified punctate diffusion-weighted imaging restricted lesions in 15% of patients who had a lobar ICH, but none in patients diagnosed with Alzheimer disease, was the first that suggested the possibility to detect microinfarcts in the setting of severe cSVD.[28] Another study identified similar rates of microinfarct detection but somehow different distributions on diffusion-weighted imaging in HTN-cSVD and CAA patients both during the acute phase of ICH and in long-term follow-up.[29] Another study that used longitudinal diffusion tensor imaging demonstrated that asymptomatic diffusion-weighted imaging lesions produce chronic local microstructural injury in patients with CAA.[30] The major pitfall of diffusion-weighted imaging to detect microinfarcts is that the lesions are only visible during the acute/subacute phases of the infarct, that is, for 7 to 14 days. The use of ultrahigh-field strength (7T) and high-resolution imaging allow the detection of cortical microinfarcts beyond the acute phase on T_1, T_2, and fluid attenuated inversion recovery MRI but the vast majority of microinfarcts still remain under the detection limits of clinical in vivo MRI.[31] The presence of cerebral microinfarcts on 3T MRIs has been associated with vascular cognitive impairment and dementia in ischemic stroke and memory clinic cohorts.[32] It should also be remembered that both potential sources of cardiac embolism and other proximal embolic sources were associated with cerebral microinfarcts, so the ideal research in this field should account for these potential etiologies before attributing all microinfarcts to cSVD.[33,34]

Markers of Global Structural Injury Related to cSVD

Cortical atrophy is an important contributor to cognitive impairment and dementia in older adults. One of the major setbacks to determine the association between cSVD and cortical atrophy have been the common co-occurrence of parenchymal Alzheimer pathology such as senile plaques and neurofibrillary tangles in patients who have brain microangiopathies, such as CAA. A recent study compared the cerebral cortical thickness between patients with hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D, a hereditary type of relatively pure CAA, without parenchymal Alzheimer pathology) and age-matched healthy controls (mean age 46.7). HCHWA-D had significantly thinner cortex when compared to healthy controls (2.31 versus 2.42 mm, P=0.006; Figure 3).[35] Similar findings of cortical atrophy were confirmed in sporadic CAA (mean age=72 and mean cortical thickness=2.17 mm) when compared to 2 separate age-matched healthy control groups (2.31 and 2.27 mm). Patients with Alzheimer disease had more pronounced cortical atrophy when compared to age-matched sporadic CAA. A physiological measure of vascular dysfunction obtained

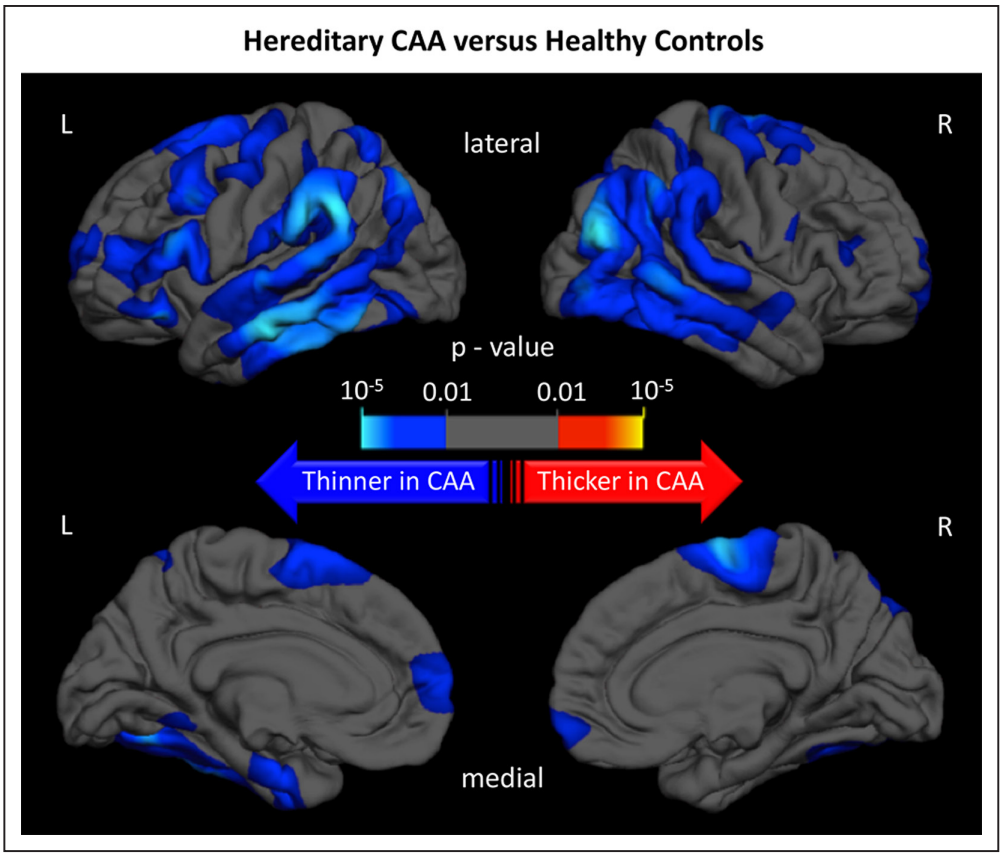

Figure 3. Topographical surface maps showing regions with significant cortical thinning in patients with a hereditary form of cerebral amyloid angiopathy (CAA) when compared with age-matched healthy controls. Reproduced from Fotiadis et al[35] with permission. Copyright ©2016, Elsevier.

using functional MRI (fMRI) methods accounted for 63% of the total effect of CAA on cortical atrophy, using mediation analyses. These fMRI measures will be further discussed under the physiological imaging section below. This study, overall, is a good example of the benefits of using advanced anatomic and physiological imaging modalities in appropriate cohorts to tackle important questions that cannot be answered with simpler designs. There is indirect evidence suggesting that hypertension might affect cortical atrophy as well, however, studies that directly test these associations in well-characterized HTN-cSVD cohorts are needed.[36,37]

Disruption of brain networks is a postulated mechanism through which cSVD can result in loss of brain function. Diffusion tensor imaging is another advanced imaging modality that provides insights into microstructural changes in brain tissue and cerebral network connectivity. Early work in the field of cSVD showed a pattern of regional brain tissue degeneration in the temporal lobe white matter and the splenium of the corpus callosum in CAA.[38] A recent advance was the use of graph theory to characterize the efficiency of the brain networks, enabling the researchers to calculate measures of global network efficiency. The use of these techniques together with amyloid positron emission tomography (PET) imaging and detailed cognitive testing showed that reduced structural brain network efficiency might mediate the relationship between advanced CAA and neurological dysfunction.[39] One potential weakness of diffusion tensor imaging is the relatively wide variability in scan parameters and the variability introduced by different analytic techniques that limit the use of this otherwise important MRI method. Recent efforts focused on developing a robust diffusion tensor imaging MRI marker based on skeletonization of white matter tracts and histogram analysis. The resultant fully automated marker, peak width of skeletonized mean diffusivity, showed excellent correlation with processing speed outperforming conventional markers (WMH, lacune, and brain volumes) in different populations, such as hereditary and sporadic cSVDs and healthy controls.[40] If these results are reproduced in the hands of independent investigators, peak width of skeletonized mean diffusivity can become an important tool in cSVD-related vascular cognitive impairment research.

Perivascular spaces (PVS or Virchow-Robin spaces) have been noted on brain imaging since the initial use of MRIs in 1980 to 1990s. Over the past years, there has been increasing number of studies that identified high numbers of enlarged PVS in cSVD patients. Specifically, high counts of enlarged PVS in centrum semiovale were more common in CAA, whereas more basal ganglia enlarged PVS were found in HTN-cSVD.[41,42] Recent studies have shown that the use of optimized T_2-weighted pulse sequences with 7T scanners significantly improves the detection of PVS. Using such high-sensitivity scans, PVS counts were not associated with age or vascular risk factors but related to cSVD markers such as CMB counts and WMH volumes.[43] Another 7T MRI study showed that enlarged juxtacortical PVS colocalize with cortical CMBs in patients with mild cognitive impairment and Alzheimer disease, suggesting a common

underlying cause, probably CAA.[44] Overall, it is likely that research into PVS will increasingly use high-resolution MRI, allowing the investigators to better understand the real meaning of these imaging markers. There are certainly other promising advanced structural neuroimaging approaches that can be highly relevant for clinical research into mechanisms of cSVD. Such methods include advanced connectome imaging modalities, quantitative susceptibility mapping, and others. They will not be reviewed as their application to cSVD-related research has not yet been established.

Molecular Neuroimaging in cSVD Research

A detailed review of molecular neuroimaging in vascular cognitive impairment, that included data relevant to the field of cSVD research, was published in *Stroke* in 2016.[3] For this reason, the fundamentals of molecular neuroimaging will only be briefly discussed, and this section will focus on advances that happened since 2016. The introduction of Pittsburgh Compound B (PiB) as a PET tracer capable of labeling brain amyloid deposits revolutionized dementia research.[45] The initial steps that led to the adoption of amyloid PET imaging by cSVD researchers involved proof-of-concept that PiB also labels vascular amyloid.[46,47] These early studies were not designed to evaluate the value of PiB for CAA diagnosis, and PiB itself was never meant to be a commercially available diagnostic tracer—due to its short half-life (≈20 minutes) requiring an onsite cyclotron and a radiochemistry laboratory with expertise in the synthesis of ^{11}C. Development of ^{18}F amyloid PET tracers, and their Food and Drug Administration-approval as potential tools for Alzheimer disease, made it possible to evaluate them as methods to diagnose CAA that could become widely available. There are some major differences in study designs that aim to validate an amyloid imaging marker for CAA diagnosis versus Alzheimer disease. The studies that validated 18F products for Alzheimer disease diagnosis were conducted in older participants with a relatively short expected survival, in whom the ultimate gold standard was full histopathologic evaluation on autopsy. Many of these patients had dementia, and this approach is totally understandable when the purpose is to compare in vivo amyloid PET imaging results to autopsy data. The situation is very different when the objective is to understand the diagnostic value of an amyloid PET tracer for CAA. This diagnosis is typically made in patients over 55 years of age who had a lobar ICH. In survivors of lobar ICH, the diagnosis of probable CAA is made based on clinical-radiological Boston criteria. Because the goal is to obtain proof-of-concept that the tracer binding mainly represents vascular amyloid, patients with dementia and mild cognitive impairment are ideally excluded, to lower the degree of confounding by parenchymal amyloid plaques. Patients with any CMB in deep locations in addition to cortical ICH/CMBs are likely to have HTN-cSVD and therefore missing such deep CMBs either because of scan parameters or investigator inexperience would also result in erroneous inclusion of non-CAA subjects into the CAA group. Finally, it is well-known that about 15% to 25% of cognitively normal older people have amyloid-positive PET scans, a condition known as presymptomatic Alzheimer disease. Although severe involvement is not common, many patients with CAA have mild degree of parenchymal Alzheimer pathology. In that sense, the enrollment criteria would also make a major difference as cognitively healthy lobar ICH survivors with multiple strictly cortical CMBs probably represent a relatively pure CAA cohort, whereas patients with microbleeds enrolled in memory clinics have probably other confounders. All of these points are important to keep in mind when critically reviewing amyloid PET studies in the field of cSVD and CAA in particular.

The first such study aimed to assess the utility of Florbetapir, a commercially available 18F amyloid tracer, in differentiating CAA from HTN-cSVD.[48] High-resolution susceptibility-weighted imaging was acquired in all 10 patients with CAA and 9 patients with HTN-ICH to minimize the risk of missing CMBs in unexpected locations. Patients diagnosed with probable CAA using Boston criteria had a median of 53 strictly cortical CMBs (interquartile range, 11–134). Patients with probable CAA and HTN-ICH survivors had same mean age (67) and similar risk factor profile. Mini-mental state score was 29 to 30 in all patients, so it was a cognitively normal group. The mean global cortical Florbetapir retention was significantly higher in CAA patients (mean standardized uptake value ratio, SUVR±SD=1.41±0.17) when compared to HTN-ICH (SUVR±SD=1.15±0.08, $P<0.001$). Using a validated binary method to categorize scans as Florbetapir positive versus negative, all CAA patients had a positive scan but only 1 out of 9 HTN-cSVD patients was amyloid positive (Figure 4). Two investigators that assessed all PET scans, blinded to other imaging/clinical data, had full interrater agreement for amyloid ± status. Patients with CAA also had PiB-PET scans and the global tracer retention correlated very strongly between Florbetapir and PiB ($r=0.96$, $P<0.001$). Overall, the study provided Class II evidence that Florbetapir PET provides 100% sensitivity and 89% specificity for determination of probable CAA in cognitively normal patients within the appropriate context.[48] Another study compared a cohort of probable CAA patients diagnosed with modified Boston criteria (only 80% with lobar microbleeds, two-third with cortical superficial siderosis) to HTN-ICH, in the acute phase of ICH without cognitive testing. This study again showed significantly higher mean global SUVR in patients with CAA compared with HTN-ICH (1.27±0.12 versus 1.12±0.12, respectively, $P=0.001$).[49] Interestingly, the sensitivity (0.6) of the visual assessment was low despite similar specificity (0.89) of Florbetapir for discriminating patients with CAA from HTN-ICH in this study. It will not be possible to rule out the probability that there might have been patients misclassified into the CAA category and that Florbetapir PET revealed more correct results than MRI-based criteria in this study but overall, the current data do not allow making clear generalizations about benefits of amyloid PET imaging for CAA diagnosis. The observed discordance also provides a cautionary tale about problems with in vivo CAA diagnosis in different cohorts using the modified clinical-radiological Boston criteria. A detailed discussion of these issues is provided in the above paragraph. More recent research shed light into some of these problems.

Two very recent studies compared the clinical and imaging characteristics and PiB-PET results of patients with ICH and CMBs in both deep and lobar locations (mixed-location

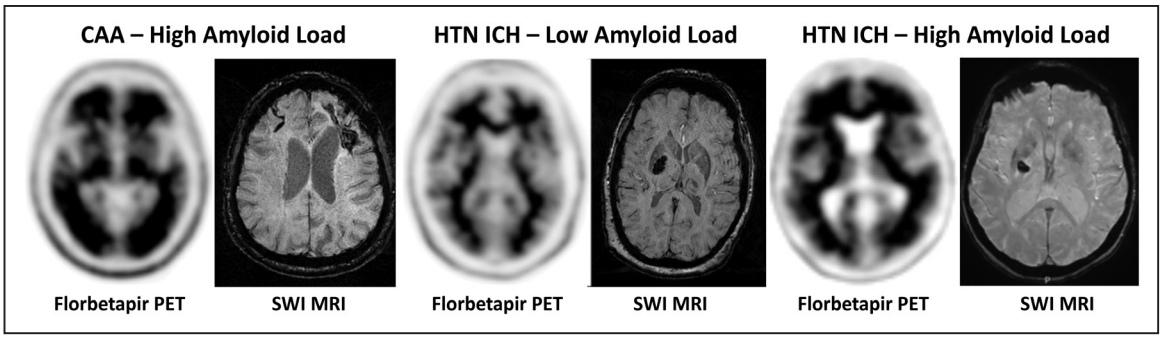

Figure 4. Illustrative examples of cerebral amyloid angiopathy (CAA) with a positive Florbetapir scan (no contrast between cortex and white matter confirming high cortical amyloid load) and negative scan (clear contrast between cortex and white matter, low cortical tracer uptake) in a patient who had hypertensive deep intracerebral hemorrhage (HTN-ICH). Last pair of scans shows a patient with deep HTN-ICH but positive amyloid scan, a false positive for CAA diagnosis. MRI indicates magnetic resonance imaging; PET, positron emission tomography; and SWI, susceptibility-weighted imaging.

ICH/CMB) separately to strictly lobar ICH/CMB (CAA) and strictly deep ICH/CMB (HTN-cSVD) patients.[50,51] The novel clinical-radiological category, Mixed-location ICH/CMB, makes up 20% to 58% of all primary ICH patients in predominantly white and Southeastern Asian populations, respectively. Clinical, laboratory and MRI features suggested a more severe form of HTN-cSVD as the predominant pathology in patients who have ICH/MBs in both deep and cortical/lobar locations. Amyloid imaging also confirmed significantly higher PiB retention in CAA (mean SUVR=1.43) when compared to both mixed-location ICH/CMBs (mean SUVR=1.06, P=0.003) and deep HTN-ICH/CMBs (mean SUVR=1.1, P=0.002).[51] These recent studies demonstrate the value of using amyloid PET imaging as a surrogate for the CAA pathology. Previous studies showed that CAA-related cortical ICH/CMBs originate from sites with higher baseline vascular amyloid deposits, a finding that was later confirmed in elegantly designed animal studies.[52,53] Human studies using PiB-PET also confirmed probable cause-effect relationships between vascular amyloid load and WMH, lobar lacunes as well as structural network alterations in patients with CAA.[26,39,54] It is probably safe to say that the use of molecular imaging will continue to be an invaluable tool to understand the associations between the molecular changes and disease processes involved in cSVD.

Physiological Neuroimaging

As discussed extensively in the respective sections of this text, cSVDs cause not only hemorrhagic but also significant ischemic consequences. Even if occlusion of individual small vessels might explain the lacunar infarcts and microinfarcts, the more widespread pathological changes (leukoaraiosis, atrophy, and decreased connectivity) are more likely related to vascular dysfunction, that is, decreased vessel reactivity. Most of the existing data again come from advanced neuroimaging studies that focused on CAA. The first-in-human proof-of-concept study compared arterial flow responses in patients with CAA to healthy controls using functional transcranial doppler. CAA patients showed a blunted increase in mean PCA flow velocities in response to a visual stimulus (8.0±6.1% versus 17.4±5.7%, P=0.002).[55] Lower visual evoked mean flow velocity increase correlated with higher CMB counts and WMH volume. The PCA pulsatility index, a marker of distal vascular resistance, was also higher in CAA than healthy controls. The reported differences between patients with CAA and healthy controls, and the correlations of the flow velocity changes with markers of disease severity, suggested the presence of vascular dysfunction in CAA, which might mediate ischemic injury.

As functional transcranial doppler has significant limitations in terms of operator dependence and the ability to measure flow velocities only in the larger vessels in and around the circle of Willis, a functional MRI paradigm and analysis approach were developed to characterize temporal features of blood-oxygenation-level-dependent (BOLD) responses to a visual stimulus to obtain estimates of vascular reactivity from the occipital cortex. Here, techniques originally designed for measuring neuronal activity through tracking changes in blood flow and oxygenation were repurposed for the measurement of impaired vascular reactivity. BOLD-weighted time-series data of subjects' block responses were fit to a trapezoidal function with parameters to describe the time to reach peak response, the response amplitude, and the time to return to baseline. The 25 patients with CAA had significantly lower amplitude as well as prolonged time to reach peak and time to return to baseline when compared to 12 healthy controls.[56] Absolute resting blood flow in visual cortex obtained using arterial spin labeling MRI was identical between the groups, again suggesting that decreased vascular reactivity is the predominant physiopathological alteration rather than a static decrease in perfusion. Within the CAA group, longer time to reach peak values also correlated with higher WMH volume, again suggesting that vascular dysfunction can mediate CAA-related ischemia. The findings of decreased amplitude of BOLD response in CAA and its association with WMH volume were reproduced in a separate cohort that used a comparable fMRI approach.[57] This second study also included visual evoked potentials and the lack of difference in visual evoked potential P100 amplitudes between CAA and controls suggested that CAA-related structural changes may not influence the propagation of the neuronal signal resulting from a visual stimulus. The fMRI paradigm that includes a visual stimulus and modeling of the BOLD response from occipital cortex has been used in multiple CAA studies to date. As discussed under the structural imaging section above, this paradigm established vascular dysfunction as the mediator of ischemic change culminating into cortical atrophy in CAA.[35] Use of the same fMRI paradigm also helped demonstrate worse

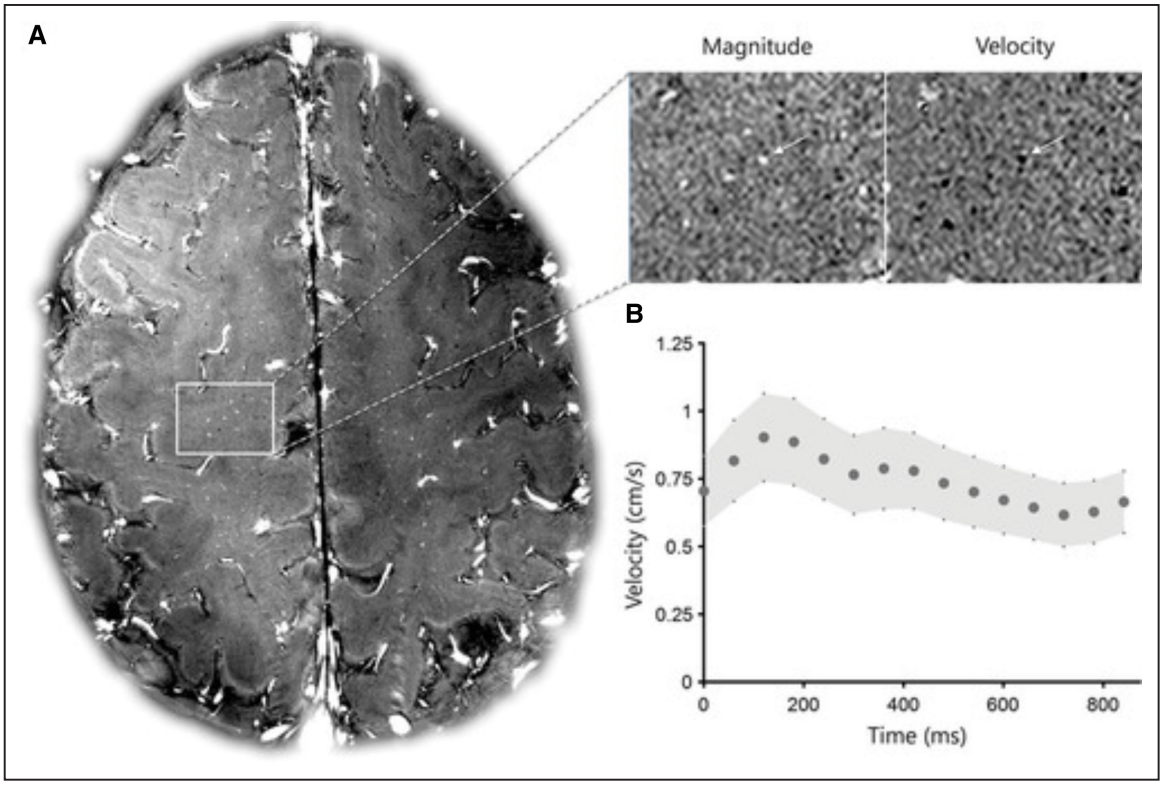

Figure 5. Blood flow velocity measurements in the centrum semiovale white matter perforators of a healthy human subject, obtained with high-resolution 2-dimensional phase-contrast magnetic resonance imaging (MRI). **A**, Mean magnitude image obtained over the cardiac cycle shows the perforators as hyperintense dots. Enlarged detail: magnitude and corresponding velocity map at single cardiac time point. **B**, Mean velocity-time curve over the cardiac cycle (average over all detected perforators [n=55]) and corresponding 95% CI. Maximum and minimal flow over the cardiac cycle can be used to calculate pulsatility index, a potential marker of small vessel stiffness. Reproduced from Zwanenburg and van Osch[60] with permission. Copyright ©2016, Wolters Kluwer Health, Inc.

vascular reactivity in symptomatic compared to presymptomatic hereditary CAA patients.[58] The rising slope of the BOLD response, calculated from the time to reach peak and amplitude based on this fMRI paradigm, also served as the main outcome measure in a Phase 2, randomized, double-blind trial of a monoclonal antibody against $A\beta_{1-40}$ (Ponezumab) to improve vascular dysfunction in CAA.[59] Although the study did not meet the prespecified efficacy criteria, the results obtained using this fMRI approach suggested that it is feasible to use the technique in a consistent manner across multiple study timepoints and sites.

A recent major advance in the field was the use of phase-contrast MRI at 7T to measure time-resolved blood flow velocity at high spatial resolution in the cerebral perforating arteries at the level of the centrum semiovale and the basal ganglia (Figure 5).[60,61] To our knowledge, these submillimeter perforators have not been targeted in any prior physiological MRI study. Using single-slice, 2-dimensional velocity-encoded phase-contrast MRI, the authors compared the number of detected arteries, the pulsatility index and the mean flow velocities between the patient groups (lacunar infarction and deep HTN-ICH) and healthy controls. At the level of basal ganglia and centrum semiovale, both patient groups with symptomatic cSVD had decreased counts of detected perforators and higher pulsatility indices when compared to controls. No velocity differences were observed. This novel physiological MRI approach holds the promise to record flow velocities from submillimeter deep perforating arteries of the brain, allowing assessment of vascular function from vessels that have never been studied in living humans previously (Figure 5). Extensions of this technique to measuring pulsatility indices from parenchymal microvasculature are currently under development.

In addition to the methods briefly reviewed above, other studies that involve physiological stimuli, such as CO_2 challenges, are commonly used to assess cerebrovascular reactivity. The incorporation of these techniques into cSVD research will increase the mechanistic yield of the studies.

Conclusions

Advanced structural, molecular, and physiological imaging modalities have increased our ability to study the mechanisms of cSVDs in living individuals. Implementing and deploying these methods into carefully designed clinical studies will increase the yield in terms of diagnostic accuracy, risk stratification, and identification of new treatment targets.

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