SUPPLEMENTAL MATERIAL

MRI-manifestations of SVD

Novel MRI-markers

DTI-based microstructural changes: have more recently emerged as novel measurements sensitive to SVD. Peak width of skeletonized mean diffusivity measures the width of the peak of the histogram of mean diffusivity after projection onto the white matter skeleton(31). Peak width of skeletonized mean diffusivity has consistently been associated with slower processing speed and WMH volume across different SVD populations(31–33). White matter free water fraction assessed with bi-tensor model fitting of conventional DTI data quantifies the amount of extracellular water(34). SVD has been associated with higher free water fraction(35), and increased free water fraction appears to precede WMH(6). Lower fractional anisotropy and higher mean diffusivity have also been associated with SVD(35), but when derived from single tensor model fitting some or most of this association may be due to increased free water fraction that cannot be separated in a single tensor model(37). In addition, SVD has been shown to disrupt structural connectivity networks mapped by means of white matter tractography(38,39).

Blood-brain barrier leakage / contrast enhancement: SVD has also been linked to increased blood-brain barrier permeability(40) which is typically assessed using dynamic contrast enhanced MRI(41). Other techniques to assess leakage include enhancement of the walls of leptomeningeal blood vessels or perivascular spaces on post-contrast FLAIR images(13). Diffusion-weighted arterial spin labeling perfusion MRI techniques have recently been developed to assess blood-brain barrier permeability without the use of exogenous contrast agents, but instead by using magnetically labeled water as an endogenous tracer to separate the fraction of water in capillaries from the fraction of water in tissue(42,43). Since these reflect relatively recent developments, the clinical relevance and relationship with cognition, as well as the neuropathological basis of these findings remains poorly understood.

Post-mortem MRI

A considerable amount of the work aiming at explaining the link between sporadic SVD and various MRI metrics requires combination of MRI and neuropathological examination in the same older adults. This can be accomplished using either in vivo MRI proximate to death or ex vivo MRI. In this section we discuss the advantages and limitations of ex vivo MRI in studies combining MRI and neuropathology, provide an overview of the evolution of ex vivo MRI, and provide our recommendations for future research.

One of the main advantages of combining ex vivo MRI with neuropathological examination is that both are conducted after death, which means that both ex vivo MRI and neuropathological examination evaluate the brain in the same condition, i.e. with the same amount of pathology and brain abnormality. In contrast, when combining in vivo MRI and neuropathology, disease progression is continuing after MRI data collection and additional pathological alterations may develop in the time interval between MRI and death(46). The

limitations related to ante-mortem intervals, which often vary between individuals, are eliminated with the use of ex vivo MRI.

Another major advantage of ex vivo over in vivo MRI is the enhanced experimental flexibility. Long acquisition times and the use of high magnetic field strengths that may not be appropriate or available for in vivo MRI of the human brain make it possible to collect data with high spatial resolution(47). In addition, ex vivo brain MRI can be conducted using special 3D printed holders that facilitate more accurate co-registration of MRI with histology images, allowing highly detailed investigation of the links between neuropathologies and brain characteristics (Supplementary Figure II).

A third advantage of ex vivo MRI is that it allows imaging independent of frailty level. Frail individuals are typically underrepresented in MRI research, which means that in vivo MRI studies on older adults may be missing an important portion of the older adult population.

Motivated by one or more of the factors mentioned above, several studies in the early days of MRI, approximately thirty years ago, used ex vivo brain MRI and histopathology to understand the histological characteristics of tissue appearing hyperintense on T2-weighted imaging(48). Because most of the MRI properties of the brain are altered post-mortem, it was important to first characterize the changes that occur in brain MRI properties to inform the development of appropriate MRI protocols tailored for ex vivo imaging. For example, it was demonstrated that T1, T2, T2* relaxation constants (49,50), and diffusion coefficients(51) of brain tissue immersed in fixative solution decrease dramatically after death and the rate at which these changes occur varies for different MRI properties. It was also demonstrated that at least in the first weeks to months post-mortem, brain MRI properties continue to change in unique ways. In addition, it was shown that factors like the temperature of the tissue must be carefully controlled(52), and that the post-mortem interval before fixation must be minimized(53). It thus became clear that the ways in which brain MRI properties change post-mortem are very much dependent on the experimental setup and brain tissue handling, and it was therefore established that ex vivo MRI requires good control of experimental parameters.

Despite the added complexity, determining how brain MRI properties change post-mortem has allowed researchers to optimize FLAIR ex vivo and generate reliable maps of WMH(54). T1 and T2 contrast can now be optimized ex vivo to allow accurate brain volumetry/morphometry(55) and to visualize lacunar infarcts, microinfarcts, and enlarged perivascular spaces(26,56). T2* contrast can be optimized ex vivo to image microbleeds, cSS, and to map magnetic susceptibility(47,57–59). Anisotropic diffusion can be assessed ex vivo, and mapping brain connections and structural brain networks is feasible(60–62). It has also been shown that several quantitative metrics of brain MRI characteristics have values ex vivo that are either similar or linearly linked to those in vivo(46,55,59,63), thereby allowing translation of ex vivo findings. Therefore, for all the reasons presented in this section, and despite the added complexity, ex vivo MRI can play an important role in research of SVD.

Neuropathology of small vessel disease MRI-manifestations

Novel MRI-markers

DTI-based microstructural changes: whereas the histopathological correlates of WMH are heterogeneous, changes in DTI metrics (i.e. fractional anisotropy, mean diffusivity) are believed to be more specific. Insights into the histopathological correlates of fractional anisotropy and mean diffusivity have mainly come from animal studies, and pioneering ex vivo DTI studies in post-mortem human brain tissue of multiple sclerosis cases(106). To the best of our knowledge, only one study to date has assessed the neuropathology associated with DTI changes in cases with SVD(61). Utilizing a previously developed MRI sequence optimized for ex vivo diffusion imaging(62), white matter tracts were successfully reconstructed in intact formalin-fixed hemispheres of neuropathologically-confirmed CAA cases(61). Fractional anisotropy was independently associated with tissue rarefaction and axonal density, whereas mean diffusivity was independently associated with myelin density(61). Notably, neither fractional anisotropy nor mean diffusivity was correlated with the number of oligodendrocytes or GFAP-positive astrocytes in examined areas. Interestingly, the study also revealed an association between increased mean diffusivity in white matter areas with increased CAA severity in the frontal cortex, suggesting a potential role for Wallerian degeneration in the formation of white matter changes(61). Important knowledge gaps include the pathophysiology underlying DTI changes, and their relationship with small vessel abnormalities in the white matter. Other fundamental unknowns include the contribution of blood-brain barrier leakage, ischemia, and neuroinflammation and whether similar mechanisms that are implied in the formation of WMH also play a role in the development of DTI alterations. Other emerging diffusion-based metrics awaiting histopathological validation include peak width of skeletonized mean diffusivity and white matter connectivity.

Blood-brain barrier leakage / contrast enhancement: there is an urgent need for neuropathological validation studies of contrast extravasation in human brain tissue to understand the histopathological basis and associated vessel and tissue changes of these emerging metrics for SVD pathophysiology. Such studies are so far lacking in SVD. Of interest is a recent report of two multiple sclerosis cases that came to autopsy after having received in vivo post-contrast FLAIR imaging(107). Histopathology in areas with leptomeningeal vessel enhancement on post-contrast FLAIR revealed perivascular lymphocytic and mononuclear infiltration overlying cortical areas with demyelination characteristic for multiple sclerosis lesions(107). The mechanism of leptomeningeal enhancement is likely different in the context of SVD. It has been suggested that it reflects leakage of plasma proteins (e.g. fibrin, IgG) and may be an early marker in the pathophysiology of hemorrhagic lesions including cSS(80,108), awaiting future studies.

Supplementary Table I. Summary of neuropathological changes underlying conventional MRI-manifestations of small vessel disease.

MRI-manifestation	**Histopathological substrates**
White matter hyperintensities	White matter rarefaction with loss of myelin, variable loss of axons, and oligodendrocytes; reactive astrocytes; activated microglia, and macrophages; enlarged perivascular spaces
Lacunar infarcts	Cystic cavities with variable macrophages, and a rim of reactive astrocytes
Microbleeds	Extravasated intact or lysed red blood cells (acute phase); biliverdin, hematoidin, and iron (subacute phase); hemosiderin-containing macrophages (chronic phase)
Microinfarcts	Tissue pallor and eosinophilic neurons (acute phase); infiltrating macrophages, reactive astrocytes, and tissue necrosis / loss (subacute phase); small fluid-filled cavities with few macrophages and a rim of reactive fibrillary astrocytes (chronic phase)
Perivascular spaces	Abnormally enlarged fluid-filled Virchow-Robin spaces surrounding arterioles
Cortical superficial siderosis	Iron-positive hemosiderin-containing macrophages in subarachnoid space and superficial layers of the cortex

Supplementary Box I. Overview of outstanding questions / knowledge gaps.

White matter hyperintensities

- What are the independent and overlapping contributions of CAA, arteriolosclerosis, amyloid β plaques, and tau tangles in the formation of WMH?
- What are the histopathological correlates and pathophysiological mechanisms of distinct WMH patterns, such as multi-spot and posterior dominant?

Microbleeds

- What are the histopathological correlates and pathophysiological mechanisms of microbleeds in the white matter and deep areas of the brain?
- What are the pathophysiological mechanisms contributing to the formation of mixed microbleeds?

Microinfarcts

- What are the histopathological correlates and pathophysiological mechanisms of microinfarcts in the white matter and deep areas of the brain?
- Are particular segments of the microvasculature more susceptible to ischemia than others?

Perivascular spaces

- What are the histopathological correlates and pathophysiological mechanisms of MRI-visible perivascular spaces in deep areas of the brain?
- Do enlarged perivascular spaces mainly occur around arterioles, venules, or both?
- Do enlarged perivascular spaces affect the integrity of the white matter beyond the spaces? And if so, through which mechanisms?

Cortical superficial siderosis

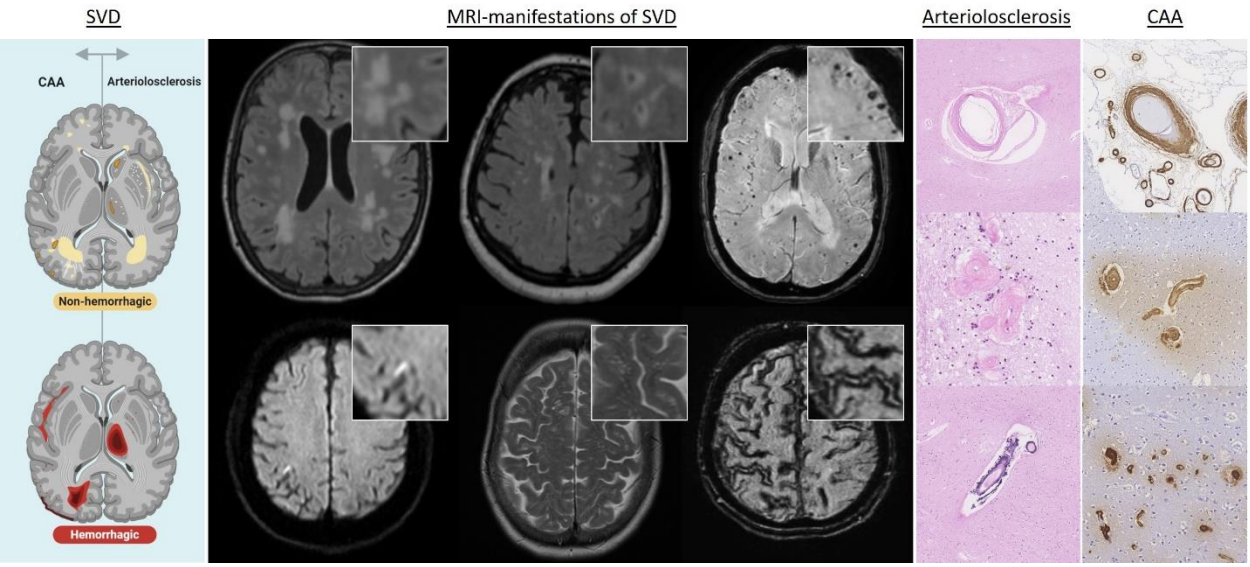
- Do similar mechanisms of vascular remodeling and amyloid β removal that appear to play a role in the formation of cortical microbleeds also occur in leptomeningeal vessels involved in cSS?
- What are the secondary tissue changes and pathophysiological mechanisms responsible for increased bleeding risk after cSS in patients with CAA?

DTI-based microstructural changes

- What are the pathophysiological mechanisms underlying altered DTI metrics (i.e. fractional anisotropy, mean diffusivity)?
- What are the histopathological correlates and pathophysiological mechanisms of peak width of skeletonized mean diffusivity and white matter connectivity changes?

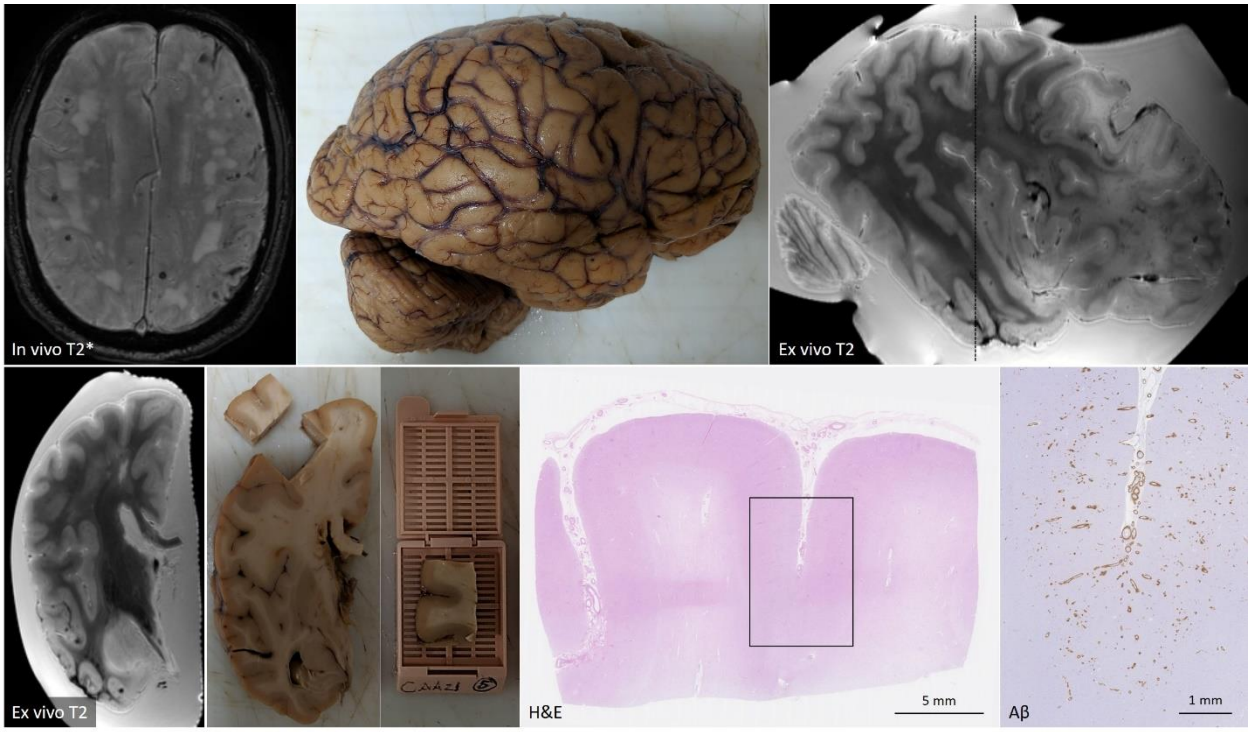
Blood-brain barrier leakage / contrast enhancement

- What are the histopathological correlates and pathophysiological mechanisms of dynamic contrast enhanced changes in the brain parenchyma?
- What are the histopathological correlates and pathophysiological mechanisms of leptomeningeal enhancement on post-contrast FLAIR images?



Supplementary Figure I. Representative examples of SVD on neuropathology and SVD on MRI.

A schematic representation of parenchymal brain abnormalities with their typical topographical predilections in patients with sporadic SVD is shown on the left. Representative examples of MRI-manifestations of sporadic SVD are shown in the middle, and include (from left to right, top to bottom): WMH, lacunar infarcts, microbleeds, microinfarcts, enlarged perivascular spaces, and cSS. Representative examples of CAA and arteriolosclerosis on standard neuropathological examination are shown on the right. Prominent features in both types of SVD include thickening and fragmentation of the vessel wall. Note: the panel with the schematic representation of SVD (left) is adapted from Zanon Zotin et al. (109) with permission, the panel with the MRI-manifestations of SVD (middle) is adapted from Kozberg et al. (110) with permission.



Supplementary Figure II. Representative example of an in vivo MRI – ex vivo MRI – neuropathology pipeline.

Typical stages involved in ex vivo MRI studies include for example (from left to right, top to bottom): an ante-mortem MRI scan (depicted here is an in vivo T2*-weighted scan in a patient with a clinical diagnosis of probable CAA), a formalin-fixed intact hemisphere (from the same individual), a high-resolution ex vivo MRI scan (depicted here in sagittal and coronal orientation), the co-registered formalin-fixed cut brain slab, sampling in a standard tissue cassette followed by paraffin embedding, cutting and staining with standard histopathological and immunohistochemical techniques (depicted here are an H&E- and adjacent amyloid β-stained section).