Advanced MR image analysis in sporadic and Dutch-type hereditary Cerebral Amyloid Angiopathy

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Chapter 8 | Summary, general discussion, and future perspectives

Summary

Advanced magnetic resonance (MR) image analysis provides insight in pathophysiological processes and disease evolution in patients with Cerebral Amyloid Angiopathy (CAA), that cannot be derived from CAA-related markers on conventional MR imaging. Markers that rely on advanced MR image analysis are discussed in this thesis, covering the range of MRI-measurable stages from the proposed CAA pathophysiological framework[8]. In part I, cerebrovascular reactivity (CVR) changes as measured with visually stimulated functional MRI (fMRI) were studied, both in relation to microstructural white matter integrity (Chapter 2) and longitudinally with one-year follow-up data (Chapter 3). In part II, perivascular spaces (PVS), the proposed highways for brain clearance, were quantified cross-sectionally and longitudinally with a newly introduced pipeline (Chapter 4). Part III focuses on localization and clustering of cerebral microbleeds (CMBs) and the occurrence of acute CMBs, with Chapter 5 covering the distribution of CMBs along the curvature of the cortex, Chapter 6 investigating interindividual clustering of CMBs with a flow territory-based analysis, and Chapter 7 focusing on the occurrence of acute CMBs as recognized on 7 Tesla (T) MRI.

Part I – Cerebrovascular reactivity measured with visually stimulated functional MRI

In Chapter 2 we assessed the relationship between CVR changes, as measured with visually stimulated Blood-Oxygen-Level-Dependent (BOLD) fMRI, and microstructural white matter integrity, as measured with the relatively novel and robust measure peak width skeletonized mean diffusivity (PSMD) based on diffusion tensor imaging (DTI), in patients with Dutch-type CAA (D-CAA). We found: 1) an association between BOLD amplitude reductions of the vascular response and deteriorated microstructural white matter integrity, once the vascular response is severely impaired (unstandardized $\beta = 0.64$, 95% CI [0.10, 1.18], $p = 0.02$, Adjusted $R^2 = 0.48$) and 2) an association between a delayed vascular response and deteriorated microstructural white matter integrity (for prolonged time-to-peak (unstandardized $\beta = 8.34 \times 10^{-6}$, 95% CI [$1.84 \times 10^{-6}$, 1.48×10^{-5}], $p = 0.02$, Adj. $R^2 = 0.25$) and prolonged time-to-baseline (unstandardized $\beta = 6.57 \times 10^{-6}$, 95% CI [$1.92 \times 10^{-6}$, 1.12×10^{-5}], $p = 0.008$, Adj. $R^2 = 0.29$)). These results indicate that a delay in vascular response might go hand in hand with microstructural white matter deterioration, whereas a decrease in the strength of the vascular response is only related to microstructural white matter damage in a later stage. It appears that a delayed and decreased blood supply affect the microstructural white matter integrity, but there is relative preservation at first, likely due to the relatively low metabolic demand and collateral blood supply of the white matter[234].

The follow-up assessment of CVR in (D-)CAA, as assessed with visually stimulated BOLD fMRI, showed in Chapter 3 that deterioration of the vascular response after one year was limited in the current study sample. While the amplitude and time-to-peak of the BOLD response showed substantial variation, a clearer deterioration was visible when combining both parameters into the upslope (percentual BOLD amplitude change divided by time-to-peak). The variability and lack of clear deterioration of the parameters is likely due to the advanced disease stage of the participants in the study sample. Changes in the vascular response to a visual stimulus has been shown to be the first MR marker to be affected in (D-)CAA, therefore, sensitivity to worsening of the vascular response might be more pronounced in earlier disease stages, compared to the later stages[8, 20].

The findings on CVR changes in (D-)CAA enhance our understanding of early disease processes. By studying the relation between BOLD CVR changes and microstructural integrity, we aimed to disentangle the relation between proximal and more distal effects of vascular amyloid-beta (Aβ) accumulation. Observations of microstructural white matter integrity loss once there are severe reductions in the strength of the vascular response is in line with the proposed temporal ordering of CAA, which proposes first vascular Aβ deposition, followed by reduced CVR, then non-hemorrhagic brain injury, and lastly hemorrhagic lesions[8]. Combining multiple parameters, in this case to calculate the upslope, may enhance interpretability of otherwise ambiguous results. Going forth, considering the upslope in studying BOLD fMRI parameters is recommended.

Part II – Quantification of perivascular space volume

In Chapter 4 we have introduced a novel pipeline for performing whole-brain white matter semi-automated PVS segmentations on T2-weighted images, which is especially fit to overcome limitations that other methods encounter in the presence of disruptive pathology. In short, our pipeline focused the region of interest (ROI) for PVS segmentation on the normal appearing white matter of the cerebrum, excluding e.g. white matter hyperintensities (WMH) and intracerebral hemorrhages (ICH). The ROI was initially based on white matter segmentations and manual adjustments were made to ensure coverage of only the normal appearing white matter and exclude other white matter segmentation inaccuracies. Subsequently, the Frangi vesselness filter was applied in an interactive viewer that allowed for inter-subject optimization of the Frangi vesselness filter threshold and settings. With this method, we replicated previously found increased levels of EPVS in symptomatic D-CAA[25] (in comparison to controls ≤ 50 years ($p < 0.0001$, 95% CI [-0.051, -0.025]) and to controls > 50 years ($p < 0.0001$, 95% CI [-0.042, -0.016])) and extended these findings to whole-brain white matter PVS quantification. In addition, we found increased PVS volume fractions in the early, presymptomatic, stage of D-CAA (in comparison to controls ≤ 50 years ($p = 0.023$, 95% CI [-0.035, -0.002])). Lastly, we found no group-level changes in PVS volume fraction over four years of follow-up when comparing D-CAA mutation carriers to control participants ($F(3, 9) = 1.183$, $p = 0.369$). However, when examining PVS volume fraction as function of age, we observed a clear increase in PVS volume fraction from approximately 40 to 60 years of age. From 60 years onwards, we observed a decrease in PVS volume fraction. This might be explained by 'loss' of regions with the highest underlying pathology, and thus conversion of regions with the highest levels of (susceptibility for) PVS to WMH or ICH.

Part III – Cerebral microbleeds localization, clustering, and the acute hemorrhagic phase

Research discussed in Chapter 5 showed an increased CMB density in the sulcal cortex as compared to the gyral cortex in (D-)CAA ($B = 0.618$, $\exp(B) = 1.86$, $p < 0.001$). Our findings indicate variations in hemorrhagic vulnerability along the cortex, which may arise from anatomy-based variations in inflammation and cerebrospinal fluid (CSF) flow dynamics. A potential mechanism could be lower CSF flow or movement in sulci than along the gyri, where there might be more free CSF flow. This could result in waste products, like Aβ, to get more trapped in sulcal depths. In addition, it may cause inflammatory cells to linger, thereby disrupting the local environment.

In Chapter 6 we showed that there is interindividual clustering of CMBs in (D-)CAA when using

a flow territory-based analysis (χ^2 = 6,920, p < 0.001). Highest CMB densities were observed in the right posterior cerebral artery (PCA), right middle cerebral artery (MCA), and left PCA. Additionally, on a group-level, when considering all participants, we did not observe clear lateralization patterns (e.g. CMB density in the territory of the left (PCA) did not differ from the territory of the right PCA). Our main analysis was performed in a subset of participants without symptomatic ICH (sICH), as we suspected a potential effect of sICH on CMBs patterns. As an anecdotal description: we often noticed an increased number of CMBs surrounding large ICH. To assess whether our findings were robust in the presence of sICH, we performed a sensitivity analysis in which we included all participants (also including participants with a history of sICH). This sensitivity analysis showed robust results, however with a stronger effect size (χ^2 = 102,027, p < 0.001). This may indicate that the clustering effects are more pronounced with the presence of sICH, which aligns with our previous – but not systematically assessed – observations of an increased number of CMBs surrounding sICH. Clustering patterns, also in the absence of sICH, suggest variable regional vulnerability that could be influenced by local pathology, vascular anatomy, or local disease progression. To expand upon this, one might hypothesize that brain regions with more CMBs have more severe underlying pathology – e.g. inflammation or elevated levels of accumulated vascular Aβ – and are therefore more likely to suffer from sICH. Conversely, secondary damage and inflammation typically occur with sICH, where the development of CMBs could be the result of sICH occurrence[235, 236]. However, causality and incidence of sICH near CMB clusters, or vice versa, should be studied in a longitudinal format.

In Chapter 7 we evaluated the presence of the T1 hyperintense lesions that are suspect for acute CMBs on 7T MRI. We started looking into these lesions due to incidental observations of small hyperintense spots on our 7T T1-weighted imaging. Anecdotally, for logistical reasons we typically performed the 7T MRI before the lumbar punctures on study days. To ensure the absence of acute bleeds that might pose a contraindication for a lumbar puncture, we always checked the T1-weighted images thoroughly for the presence of acute bleeds. It was during these assessments that we spotted very small T1 hyperintense lesions that showed susceptibility artefacts on 7T T2* gradient echo, indicating a hemorrhagic origin. Based on these initial observations we decided to systematically check all our cross-sectionally available 7T MRI scans for the presence of these acute CMBs to assess the prevalence in our cohort. We found a total of 16 lesions distributed over 11% of the D-CAA and sCAA study participants. We also checked the 3T MRI scans of the same participants and saw that the lesions were visible on approximately one half of the 3T MRI scans. Based on follow-up imaging, we confirmed that 11 lesions were acute CMBs at the time of first discovery. This study highlights the increased sensitivity that can be achieved with ultra-high field imaging. Although these findings have no direct clinical implications for (D-)CAA at the moment, they may aid in our understanding of the underlying mechanisms leading to CMBs.

General discussion and future perspectives

A growing focus on (Dutch-type) Cerebral Amyloid Angiopathy

Whereas CAA used to be described as one of the largest diseases you have never heard of, times have been changing. Progressively more is known about CAA; it has reached the focus of many researchers and research groups, big consortia have been set up with the main aim to study disease mechanisms and disease progression of CAA, treatment trials are running and have been run in CAA and D-CAA. Also, as more is known about its comorbidity with Alzheimer's disease (AD) – CAA is present in roughly half of the cases with AD[3] – interest in CAA seems to increase even more. In addition, recent advancements in anti-Aβ immunotherapies for AD, and the increased risk of Amyloid-Related Imaging Abnormalities (ARIA) in the presence of CAA, call for the recognition of CAA comorbidity[237-240].

A large contribution to scientific advancements in the field of CAA has been through studies that looked into D-CAA. As previously noted, the autosomal dominant inheritance pattern of the causative genetic mutation for D-CAA, enables early and definitive in vivo diagnosis, allowing the study of early disease phases. Early-phase mechanisms are of particular interest, since early interventions often yield the most substantial therapeutic benefits. Additional to being able to study the early phase, we can study disease mechanisms with fewer age-related confounders and follow participants with D-CAA over a relatively long period, enabling us to investigate disease mechanisms that occur prior to sICH – offering valuable insight into a crucial time window for the disease.

The origin of cerebrovascular reactivity reductions

Chapter 2 and 3 both cover CVR changes as measured with visually stimulated BOLD fMRI. Although visually stimulated BOLD fMRI is the gold standard to measure CVR changes in (D-)CAA, there are some important considerations when it comes to understanding and interpreting these measures.

First, the BOLD signal in response to visual stimulation is initially the result of neurovascular coupling. However, previous research using hypercapnic challenges has identified that CAA-related reductions in BOLD signal upon visual stimulation result from independent CVR changes[241]. These CVR changes in turn result in impaired neurovascular coupling. With mouse models, it has been shown that CVR is reduced in response to the endothelial-dependent vasodilator acetylcholine (in a type I CAA mouse-model) and that vasoconstriction is additionally impaired in response to induced cortical spreading depression and alpha-chloralose anesthesia (in a type II CAA mouse-model)[242, 243], again indicating that the CVR changes are independent of changes in neurovascular coupling.

Second, as BOLD fMRI is more sensitive to the venous side and is dependent on a combination of cerebral blood volume (CBV), cerebral blood flow (CBF) and oxygen consumption, it remains unclear what components have the largest contribution to the observed decreases in signal. Understanding the mechanisms of reduced BOLD fMRI signal changes will further increase our understanding of CVR changes on both a biological and mechanistic level. So far, arterial spin labeling-perfusion studies have not shown CBF to be reduced in CAA[17] and a hypercapnic challenge (with vasodilatory stimulus) has shown a decrease in vascular response, even when oxygen consumption remained unchanged[241]. These results might indicate that impairment

to modulate CBV is one of the largest contributors to the reductions in BOLD fMRI signal. However, changes in CBV, as measured through vascular space occupancy (VASO) fMRI, did not show a significant decrease in the earliest disease stages of CAA when BOLD fMRI did show changes[244]. This may partly be explained by the lower signal-to-noise ratio of VASO. Especially in the early phase of the disease, an interplay of multiple facets may result in the observed CVR changes.

Lastly, there is quite some variability in the measured vascular responses as is for example clear from our one-year follow-up study and sample-sizes have currently been relatively small. Therefore, it would be interesting to study a large dataset with a substantial age range and assess BOLD fMRI parameters, including upslope, over a longer follow-up period.

Perivascular spaces in relation to brain clearance

Early EPVS may indicate CSF stagnation and impaired clearance from PVS as an early-stage process of the disease[47]. However, PVS volume is at best just a proxy for brain clearance and we do not yet understand how PVS volume directly relates to brain clearance. Moreover, the assessment of PVS volume remains a nontrivial challenge. In Chapter 4, we have introduced a semi-automatic pipeline to quantify PVS that overcomes limitations of the gold standard PVS assessment (the visual rating scale[56]), but some considerations are worth mentioning.

First, currently available pipelines typically exclude WMH regions from PVS ROIs or assume WMHs to be non-PVS regions[57, 126]. However, when looking at T2-weighted scans, we often observe that PVS are visible within WMHs. Current PVS assessment methods are not capable of identifying and quantifying these WMH-PVS, likely because of the limited contrast between the two. Studying these PVS would provide insights on how PVS might develop in the context of white matter damage. Potentially, isolating the CSF signal using the CSF-STREAM sequence, might aid in assessing PVS that are localized within WMHs[245]. However, also with CSF-STREAM, WMH-fluid contents are expected to decrease contrast with the PVS.

Second, by considering total volume fraction, as we propose in our pipeline (Chapter 4), regional information is neglected. Regional effects might be particularly relevant when considering the mechanistic role of PVS in relation to e.g. white matter damage, cerebral hemorrhages, or morphological dynamics as a result of cortical spreading depolarization[246]. In addition, by looking at total volume fraction, number of PVS and individual PVS size, shape, and dynamics are not taken into account. However, PVS volume fraction is likely more informative than number of PVS. We have shown increased sensitivity of PVS volume fraction by identifying PVS enlargement as early disease marker in D-CAA. Moreover, a study investigating PVS across the healthy lifespan (~8-90 years old) has shown that PVS diameter is the largest predictor of PVS volume fraction in the white matter (explaining ~21% of the unique variance), followed by number of PVS and solidity (explaining ~4 and 3% of the unique variance, respectively)[247]. Number of white matter PVS seems to increase until middle age (~55-60 years of age), potentially hinting towards convergence of PVS with increasing volume, resulting in lower numbers of PVS, stressing the relevance of volume over count. In addition, PVS size increases and decreases have previously been reported, indicating the potential relevance of investigating PVS dynamics to understand what drives size fluctuations[248].

Lastly, longitudinal PVS volumetric data is currently limited and inconclusive. One study

shows an increase in white matter PVS volumes over three-years, with faster rates in AD amyloid positive participants compared to amyloid negative participants[249]. Another study shows increased but stable PVS diameters in participants who develop dementia over time and a reduction in PVS diameter in participants who do not develop dementia (median follow-up of ~4 years)[250]. In our study (Chapter 4), we do not observe significant changes in PVS volume fraction over a four-year follow-up. Varying longitudinal results may be related to the impact of other disruptive brain pathology. Areas that are expected to show the strongest increase in PVS volume fraction, are likely the areas that are most prone to convert in WMH or ICH over time, especially in CAA[47]. Propositions for future research to study longitudinal changes in depth include: 1) focusing on regions that develop into WMH or ICH over time, and assess relative regional PVS volumes, and 2) investigating individual PVS on high-resolution scans to focus on individual PVS enlargement over time, to disentangle PVS dynamics on a single-structure level.

To further explore the link between PVS volumes and brain clearance, assessing CSF-mobility, e.g. using CSF-STREAM, along a range of PVS volumes might provide functional information[245]. Also, tracking waste products that move through the CSF in PVS would provide insights on the role of PVS in brain clearance. Although this might be difficult to assess in vivo, T2-values of CSF-STREAM imaging – where lower T2-values might indicate polluted CSF – may provide some insights on this in vivo and non-invasively[245].

Cerebral microbleed localization and clustering

Localization of CMBs might help us to increase understanding of underlying disease pathology and potentially contribute to prediction of ICH location.

Considering the gyrencephalic anatomy of the human brain and the influence of arterial pulsations, aquaporin-4 water channels, and potentially even the presence of membranes in the subarachnoid space, on CSF properties, it seems intuitive that CSF flow along the curvature of the cortex might be heterogenous[44, 170, 251]. However, research into this has been very limited and has primarily been done in non-humans. Findings related to CSF flow along the cortex in humans has been limited to showing alignment of initial tracer influx along the major cerebral arteries in the larger fissures and cisternal spaces[174, 123, 175]. The role of arterial pulsations in movement of CSF in PVS – and the potential influence of alterations in arterial pulsations on waste clearance – can be directly linked to vascular Aβ accumulation and resulting vessel wall remodeling[8, 47, 53, 252]. The predominance of CMB in the sulcal cortex may reflect the hypothesized cycle of Aβ accumulation, vessel wall remodeling and rigidness, reduced CSF flow, and decreased clearance, eventually leading to CMBs. Thus, based on the results presented in Chapter 5, one might hypothesize that the sulcal cortex might be more prone to Aβ accumulation with potentially related reductions in CSF flow and clearance. In addition, inflammation may play a role in the occurrence of CMBs. Previous research has shown that CMBs are more common in CAA related inflammation (CAA-ri) compared to CAA[253]. CAA-ri also has lower CSF $A\beta_{42}$ levels, potentially hinting towards stronger impaired brain clearance in CAA-ri and suggesting the combined role of inflammation and brain clearance in the development of CMBs[253]. If the sulcal areas are indeed more susceptible to having reduced CSF flow and potentially reduced clearance, inflammatory cells may fail to clear and therefore stay relatively confined to these areas, potentially causing further disruption of the local environment which could lead to further damage and thus CMBs.

Interindividual CMB clustering patterns in presence and absence of ICH, as observed in Chapter 6, point towards interindividual variations in spatial patterns of factors associated with CMBs, such as brain clearance and inflammatory processes. Spatially varying hemorrhagic vulnerability may be an interesting avenue for further research, as it may help us in understanding and spatially localizing active disease stages.

To continue research in this line, it would be relevant to perform longitudinal studies to understand the topographical relation between CMB clusters and ICH. Further, explorative clustering analyses might help us further understand on what level clustering occurs. Lastly, topographical correlations of CMB clusters to other CAA-related MR markers, e.g. cortical superficial siderosis (cSS), may provide relevant insights for better understanding disease mechanisms and progression.

Acute cerebral microbleeds

Building upon understanding active disease stages, assessment of the occurrence of acute CMBs, as discussed in Chapter 7, might contribute by raising awareness for previously mostly unseen markers of disease activity. The fact that we observed acute CMBs in approximately 10% of our cross-sectional sample, points towards a relatively high occurrence and thus presence of active disease stages. Histological research has evidenced the neuroinflammatory role in CMBs by the presence of neuroinflammatory markers in both old and recent CMBs. Old CMB have higher levels of neuroinflammatory markers than recent CMBs, but recent CMBs are associated with increased astrocyte activation (measured as glial fibrillary acidic protein (GFAP) expression)[254]. Identification of such neuroinflammatory-related active disease stages may aid in mechanistic disease understanding.

Age correction in cerebral amyloid angiopathy research

A potentially controversial topic is statistical age correction, or the lack of age corrections. In relation to (D-)CAA, this is a topic that has often been discussed and remains a hot topic of discussion. Age corrections in (D-)CAA study samples based on (D-)CAA aging effects will often result in an underestimation of the effect size by overcorrecting for age, because of a very strong disease effect with age in (D-)CAA[11, 26, 220]. Therefore, to correct for age in a (D-)CAA sample, we should consider the 'normal aging'-effect, thus using healthy control groups. However, this can pose difficulties to study designs and participant inclusion. A potential solution would be to use externally available healthy datasets that include parameters or measures of interest, especially for the more robustly assessable markers – as we have done in Chapter 2, where we compare the robust measure of PSMD against an external healthy control cohort that clearly shows that PSMD values in D-CAA deviate from a very early age onwards and deteriorate faster with aging in (D-)CAA than with the 'normal aging'-effect. Omitting this correction based on normal aging, would have resulted in an overcorrecting for age and therefore an underestimation of the true effect size. To support the initiative of considering the 'normal aging'-effect rather than the age effect in the sample with disease, research is needed to evaluate robustness of parameters when measured using different scanners, vendors, across field strengths, as well as across diverse populations.

MRI research and post-processing

MRI research in patients with (D-)CAA has proven to be of great relevance, with regard to diagnostics, tracking of disease progression, and mechanistic disease understanding. MRI is a non-invasive and incredibly diverse imaging modality. Studies from this thesis serve as examples for the diversity of MRI-imaging; markers related to all disease stages, as proposed within the pathophysiological framework, except for the initial Aβ accumulation stage, can be visualized using MRI.

For many markers that are assessed in this thesis, post-processing fulfills a significant role. For some markers, e.g. CVR changes in response to a visual stimulus as measured with BOLD fMRI, post-processing is a necessity in order to interpret the data. For other markers, e.g. PSMD to assess microstructural white matter integrity and quantification of PVS volume fraction, post-processing enables to measure disease progression with increased sensitivity, allowing for detection of early and subtle deviations, in comparison to qualitatively assessable markers. The added value of post-processing and quantifying markers stresses the need for more research to optimize pipelines and make them easily applicable and accessible among researchers and clinicians. Such pipelines and automated scoring systems can help make markers more robustly assessable.

Concluding remarks

In conclusion, the research presented in this thesis has examined a range of MR markers with advanced MR image analyses, reflecting the MRI-measurable pathophysiological stages of (D-)CAA. To be able to understand disease processes and mechanisms as a whole, it is necessary to zoom in and assess the different components in a way that is supported by study setups. In this thesis, we have zoomed in on multiple aspects that contribute to and form part of the disease processes and mechanisms of (D-)CAA, through exploitation of the location of pathology, hemorrhagic stages, quantification of markers, image post-processing, early disease identification, cross-sectional study designs, and longitudinal scanning. Together, these aspects help to shape the picture of how, why, and when (D-)CAA commences and progresses and open up possibilities to look at dynamics of the disease and continuation of advanced imaging and analysis.