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

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Chapter 1 | General introduction

Cerebral Amyloid Angiopathy

Cerebral Amyloid Angiopathy (CAA) is a cerebral small vessel disease (cSVD) that is characterized by the accumulation of amyloid-beta (Aβ), predominantly the soluble $A\beta_{40}$, within the vessel walls of cerebral cortical and leptomeningeal arteries. Intracerebral hemorrhage (ICH) is the most well-known clinical manifestation and can lead to function loss or even be fatal. Other clinical manifestations are transient focal neurological episodes (TFNEs) and cognitive decline, including vascular dementia[1, 2]. The prevalence of the most common form of CAA, sporadic non-hereditary CAA (sCAA), is very high at advanced ages; CAA is identified in almost a quarter of the general population with brain autopsies[3]. The protein accumulation in CAA is very similar to that in Alzheimer's disease (AD), however, there is a predominant accumulation of the non-soluble form $A\beta_{42}$ in AD which is located in the parenchyma instead of in the arteries. There is a large comorbidity of CAA in AD; almost half of post-mortem AD assessed with brain autopsy have comorbid CAA[3].

Magnetic resonance imaging (MRI) has been shown to be a very important measurement modality for studying in vivo CAA as there is a large variety of CAA-related MR markers that noninvasively facilitate probable and possible diagnosis of CAA[4]. In addition, these MR markers allow us to noninvasively track disease progression, which is of importance not only for predicting disease progression but also for tracking treatment effects. Some of the most common CAA-related MR markers are cerebral microbleeds (CMBs), ICH, cortical superficial siderosis (cSS), white matter hyperintensities (WMH), WMH in a multispot pattern, and enlarged perivascular spaces in the centrum semiovale (CSO-EPVS). The probable and possible diagnosis of CAA is based on age, clinical symptoms (TFNEs, cognitive impairment, or vascular dementia), and radiological findings, via the Boston criteria v2.0 (Table 1)[4]. Definite diagnosis of CAA is only possible through full brain post-mortem examination (Boston criteria v2.0, Table 1)[4].

Dutch-type hereditary Cerebral Amyloid Angiopathy

There are multiple hereditary forms of CAA, of which Dutch-type hereditary CAA (D-CAA) is the most common and most studied variant. D-CAA is caused by an autosomal dominant point mutation at codon 693 of the amyloid precursor protein (APP) gene[5, 6]. This mutation causes the production of a malformed type of Aβ that has a higher tendency to aggregate, leading to earlier onset of D-CAA, compared to sCAA[5, 6]. However, the clinical, radiological, and pathophysiological aspects of D-CAA are very similar to sCAA, rendering D-CAA as an ideal model to study sCAA.

sCAA can be difficult to study since definitive diagnosis is only possible post-mortem and most patients only present at later disease stages[4], resulting in increased prevalence of age-related comorbidities – e.g. cardiovascular risk factors that may complicate the interpretation of CAA pathology. Studying D-CAA allows us to investigate disease mechanisms, while overcoming the previously mentioned challenges. Definitive D-CAA diagnosis is possible through genetic testing which enables identification of mutation carriers before complaints arise. In addition, the autosomal dominant inheritance pattern of the APP mutation allows identification of family lineages that are genetically predisposed. Early disease identification also allows for

Table 1. Boston criteria v2.0

Definite CAA	
	Full brain post-mortem examination, with: • ICH, cSAH, TFNE, or cognitive impairment or dementia • Some degree of pathologically determined CAA
Probable CAA with supporting pathological tissue (evacuated hematoma or cortical biopsy)	
	Clinical data and pathological tissue, with: • ICH, cSAH, TFNE, or cognitive impairment or dementia • Some degree of pathologically determined CAA
Probable CAA without pathological tissue	
	50 years of age or older Presentation with ICH, TFNE, or cognitive impairment or dementia MRI criteria: • Two or more strictly lobar T2*-weighted MRI detectable lesions in the form of ICH, CMB, cSS foci or cSAH or one lobar hemorrhagic lesions with one white matter marker in the form of severe CSO-EPVS or a multispot WMH pattern
Possible CAA	
	50 years of age or older Presentation with ICH, TFNE, or cognitive impairment or dementia MRI criteria: • One strictly lobar T2*-weighted MRI detectable lesions in the form of ICH, CMB, cSS foci or cSAH or one white matter marker in the form of severe CSO-EPVS or a multispot WMH pattern
Requirements for all diagnoses: • Absence of other diagnostic lesions (definite and probable supported with pathology) or other cause of hemorrhagic lesions (probable without pathology and possible) • Absence of deep hemorrhagic lesions (excluding cerebellar lesions)	

Abbreviations. CAA; Cerebral Amyloid Angiopathy. ICH; intracerebral hemorrhage. cSAH; convexity subarachnoid hemorrhage. TFNE; transient focal neurological episode. MRI; magnetic resonance imaging. CMB; cerebral microbleed. cSS; cortical superficial siderosis. CSO-EPVS; enlarged perivascular spaces in the centrum semiovale. WMH; white matter hyperintensities.

a distinction between the pre-symptomatic and symptomatic phase – terms that will return often in this thesis. In the pre-symptomatic phase, mild cognitive changes may be present, but no symptomatic hemorrhage has occurred that has been clinically recognized and confirmed by imaging[7]. In the symptomatic phase, such a symptomatic hemorrhage has occurred, and has been clinically recognized and confirmed by imaging.

Stages of disease progression in Cerebral Amyloid Angiopathy

Disease progression in CAA has previously been described in four sequential stages that align with vessel wall remodeling and both imaging and clinical markers[8]. The stages form a basis for understanding disease development and create a framework for CAA-related MR markers that will be discussed in the following chapters of this thesis.

Stage I – vascular amyloid-beta deposition

The first stage of the framework entails the initial vascular Aβ deposition, which coincides with decreased levels of Aβ in the CSF. Studies in D-CAA mutation carriers have shown that Aβ CSF-levels already deviate approximately 30 years before the occurrence of the first symptomatic hemorrhage[8-13]. Histopathological samples of early CAA have shown that smooth muscle cells remain relatively intact, with Aβ depositions within the outer basement membranes surrounding the smooth muscle cells[14, 15].

This initial stage is difficult to evaluate in vivo, since it requires either histopathological analysis of brain tissue of CSF analysis; with the latter only providing indirect evidence of Aβ deposition with substantial variability in Aβ values in CSF samples between patients[16]. Whereas for the other stages, MRI assessments enable non-invasive and relatively robust tracking of the disease, MRI currently has a blind eye for the first stage of CAA.

Stage II – reduced cerebrovascular reactivity

The second stage of the disease is characterized by circumferential Aβ deposition in affected vessels, with replacement of vascular smooth muscle cells[15]. This stage is expected to start approximately 20 to 30 years before the first symptomatic hemorrhage[8]. Circumferential Aβ deposition and replacement of smooth muscle cells result in reductions in cerebrovascular reactivity (CVR) changes, rendering vascular reactivity as the earliest detectable CAA-related MR marker[17-20]. Reductions in CVR have been observed in both D-CAA and sCAA through a reduced Blood-Oxygen-Level-Dependent (BOLD) amplitude and prolonged time-to-peak and time-to-baseline in response to a visual stimulus[17, 20]. Additionally, altered CVR is recognized as an early marker, since in participants with pre-symptomatic D-CAA a reduced BOLD amplitude and prolonged time-to-baseline in response to a visual stimulus is observed[20]. These observed reductions in CVR changes align with the Aβ replacement of smooth muscle cells, as the smooth muscle cells are responsible for vessel dilation and contraction.

Stage III – non-hemorrhagic brain injury

In stage three, there is circumferential Aβ deposition and complete loss of smooth muscle cells. This stage is estimated to start approximately 10 to 15 years before occurrence of the first symptomatic hemorrhage[8].

The third stage is characterized by non-hemorrhagic changes on MRI and cognitive decline. Non-hemorrhagic CAA-related MR markers include lacunes, cortical microinfarcts, WMHs, WMH in a multispot pattern, altered microstructural integrity, and CSO-EPVS. Markers that are explored in depth in this thesis are microstructural integrity and CSO-EPVS. Microstructural damage, as measured with diffusion tensor imaging (DTI), has shown impaired network

connectivity, regional (temporally located) reductions in white matter anisotropy and increased peak width skeletonized mean diffusivity (PSMD) compared to controls[21-24]. CSO-EPVS, as measured with the visual rating scale, has shown increased levels in sCAA as well as symptomatic D-CAA, with no significant progression over a four-year follow-up period[25, 26]. Previous quantification of perivascular spaces (PVS) has shown increased PVS volumes in sCAA and D-CAA (without distinction between pre-symptomatic and symptomatic disease stage) compared to healthy controls, with limited cerebral coverage[25].

Stage IV – hemorrhagic lesions

Stage four is characterized by vessel wall fragmentation, through smooth muscle cell and Aβ loss, and an inflammatory response in the form of activated astrocytes near the vessel wall[8, 27]. Both clinically and radiologically, this stage is characterized by hemorrhagic lesions due to vessel wall remodeling and can involve TFNEs.

Cerebral hemorrhages in CAA come in different forms. Lobar ICH is the most well-known marker of CAA, due to its clinical implications, e.g. function loss and fatality risk. While the mean age of the first ICH in sCAA is 72 years, the mean age of first ICH in D-CAA is as early as 54 years[28]. The annual incidence in sCAA, after the first ICH, lies around 7.4%, which is the highest ICH recurrence rate of all ICH causes. In D-CAA the recurrence rate is even higher with a hazard ratio of 2.8 compared with sCAA[1, 28]. In the general population, one-month fatality rate following ICH lies around 40%[29], and long-term mortality risk in D-CAA is higher than in sCAA (hazard ratio of 2.8)[28].

CMBs are small brain hemorrhages and can be detected on susceptibility weighted imaging (SWI) as round hypointense foci, with a diameter of the signal void up to 10 mm[30-34]. CMBs are the result of vasculopathy of the small vessels[35]. Histopathological research has shown that Aβ-levels of culprit vessels can be very low preceding a hemorrhagic event, indicating the role of vascular remodeling in the occurrence of CMB in CAA[27]. CMBs in CAA are located nearly strictly cortical and have a posterior predilection especially in the early hemorrhagic phase, but expand across the brain as the disease progresses[36-38]. The development of new CMBs usually goes clinically unnoticed, however, an increasing amount is associated with cognitive decline[39, 40].

Another type of cerebral hemorrhage in CAA is convexity subarachnoid hemorrhage (cSAH; in the acute stage) that turns into cSS when chronic. Bleeding in the subarachnoid space is thought to be the result of subtle bleeding from leptomeningeal vessels with advanced CAA[41]. Also, cSS – especially disseminated cSS – is the best predictor of future ICH[42]. cSAH and cSS can result in TFNEs – typically positive (e.g. tingling sensations in an arm) or negative (e.g. focal weakness) symptoms. TFNEs are thought to be triggered by cortical spreading depression or depolarization caused by the cSAH or cSS[43].

Cerebral Amyloid Angiopathy, brain clearance, and the role of perivascular spaces

Impaired brain clearance is the leading hypothesis for Aβ accumulation in CAA. To be more specific, Aβ-clearance is thought to be impaired in its drainage via PVS. As briefly introduced before, PVS are CSF-filled spaces between the vessel wall and astrocytic endfeet and are

mostly seen around arteries and arterioles – they are considered as potential clearance highways of the brain. Perivascular CSF motion is believed to be driven by arterial, respiratory, and vasomotor pulsations[44-46]. One possible rationale for EPVS is reduced CVR that attenuates the driving force for perivascular CSF, leading to stagnation which in turn leads to EPVS. In support of this, it has been shown that the size of PVS increases in the white matter along vessels that have Aβ accumulation in the cortical part of the vessel[47]. Another explanation might be that PVS enlarge as a coping mechanism or as mediation to increase the amount of CSF to carry waste products and potentially reduce CSF flow velocity, in order to enhance exchange with solutes from the parenchyma.

Although it has proven difficult to study the clearance process directly, especially in humans, previous findings support the clearance hypothesis: 1) decreased Aβ-levels in CSF samples obtained through lumber puncture in (pre- and symptomatic) D-CAA and sCAA as compared to controls[10], indicating Aβ is not properly cleared from the brain; 2) enlarged PVS that are associated with CAA-load in the overlying cortical portion of that vessel and with Aβ-positive Positron Emission Tomography (PET)[8, 47, 48], indicating that the enlargement of PVS – and therefore potential obstruction of the clearance pathway – seems directly associated with Aβ accumulation; 3) worsening of CAA with Aβ immunotherapy trials in AD, in which Aβ plaques are reduced, potentially leading to perivascular Aβ entrapment once it becomes soluble[49, 50]; 4) studies with Aβ-producing transgenic rodents have shown impaired tracer influx and efflux[46, 51-54], indicating that the pathways for fluid and potentially solute transport get hindered with Aβ production; 5) transgenic mice with deleted aquaporin-4 water channels show slowed CSF influx and slowed interstitial solute clearance[44, 45, 55], confirming the role of PVS as routes for clearance or transport; 6) arterial pulsations drive CSF motion and the impaired visually evoked CVR change in transgenic CAA-mimicking mice – just as we see in humans – corresponds to impaired tracer clearance along CAA-affected vessels[53], indicating CSF motion is most likely reduced in CAA once the CVR is affected.

To further understand the clearance pathways and its impairments in CAA it would be interesting to study how, where, and why PVS enlarge. In addition to contributing to our understanding of underlying clearance-related mechanisms of CAA, studying the clearance hypothesis might also contribute to the development or optimization of treatment strategies for CAA.

MR techniques within the scope of this thesis

For the MR markers assessed in this thesis, various MR techniques have been used and will be introduced here. CAA-related CVR changes can be assessed through visually stimulated functional MRI (fMRI). Visual stimulation activates the visual cortex in the occipital lobe, causing neuronal oxygen consumption and signaling the need for more oxygenated hemoglobin. In a healthy situation this results in an increase in blood flow and vessel dilation, to increase oxygenated hemoglobin levels. This increase typically overshoots the neuronal oxygen demand. The overshoot results in an increase in venous oxygenated hemoglobin, thus relatively reducing the venous de-oxygenated hemoglobin – which can be measured with MRI since de-oxygenated hemoglobin has paramagnetic properties, which dephase the MR signal. Through this principle, MRI allows us to assess CVR non-invasively.

Conventional MR images, such as fluid attenuated inversion recovery (FLAIR) and T2-

weighted imaging, allow us to assess many structural non-hemorrhagic markers. Based on T2-weighted properties we can identify PVS. However, the visibility of PVS is often limited by contrast and spatial resolution. The gold standard to assess CSO-EPVS is through a visual rating scale[56], where visible PVS are counted on a single slice in the centrum semiovale of one hemisphere. Typically, the slice and hemisphere that are estimated to have the highest amount of EPVS – often obtained from the slice with the largest volume of white matter, with minimal disruptive pathology – are selected. The counts are categorized into an ordinal scale: 0 for no EPVS, 1 for 1-10 EPVS, 2 for 11-20 EPVS, 3 for 21-40 EPVS, 4 for >40 EPVS. Limitations to this method include: a strong ceiling effect, limited coverage, and dependency on imaging field strength. Quantitative PVS assessments are available in the form of Frangi-vesselness filter-related pipelines and machine learning algorithms. However, these methods have been optimized with limited coverage and in relatively healthy brains – where no or limited disruptive pathology is present[57-62].

Detection of non-hemorrhagic microstructural damage relies on more advanced imaging. In this thesis we focus on DTI[22, 63, 64]. DTI is based on the principle of water movement via diffusion along white matter tracts. Applying MR gradients in different directions allows measurement of the degree of diffusion in these directions, resulting in three eigenvalues and eigenvectors in each voxel. The eigenvalues and eigenvectors reflect the main diffusion magnitudes and directions, respectively. Based on the eigenvalues, the mean diffusivity (MD) and fractional anisotropy (FA) can be calculated. MD provides information on the overall diffusion magnitude and FA provides information on the anisotropy of the diffusion. Higher MD values and lower FA values indicate microstructural integrity loss when looking at the white matter. Another relatively novel metric that can be assessed with DTI is PSMD[65]. PSMD is based on the distribution of MD values along the major white matter tracts that are detected through thresholding group-level averaged FA maps (the white matter skeleton). For each point on the skeleton, the maximum MD value observed perpendicular to the skeleton is back projected. The final PSMD value per participant is calculated as the difference between the 95th and the 5th percentile of these skeletally projected MD values. Thus, higher PSMD values reflect a larger spread in MD values within the major white matter tracts, indicating loss of microstructural integrity. Contamination from CSF or gray matter diffusivity, that may arise from partial volume effects, is eliminated, rendering PSMD as a robust measure for assessing microstructural integrity, especially in cSVD[66, 67].

Hemorrhages are detectable with MRI due to the susceptibility effects of hemoglobin. Gradient echo or SWI allows visualization of cerebral hemorrhages due to the signal loss that is caused by the hemosiderin deposits and the resulting blooming artefact that makes bleeds appear bigger than they are in reality. This blooming artefact is especially useful in detecting very small hemorrhages, e.g. CMBs. Temporal stages of hemorrhages can also be identified using T1- and T2-weighted imaging as hemoglobin changes over time, from the (hyper) acute (oxygenated hemoglobin) to chronic state (hemosiderin), with resulting changes in susceptibility effects[68].

Finally, 1.5 Tesla (T) and 3T are field strengths used for clinical MRIs. The use of ultra-high-field 7T MRI is typically restricted for research purposes. As a rule of thumb, the signal-to-noise ratio can be considered to increase linearly with the magnetic resonance field strength. Therefore, higher field strengths can be applied to achieve increased resolution. Moreover, higher magnetic field strengths can increase sensitivity to pathology, such as bleedings[69].

However, drawbacks of imaging with higher field strengths include potentially increased scan times, deformation artefacts, and B_1 inhomogeneities[69]. Higher field strengths are promising specifically for imaging smaller structures, e.g. for imaging PVS, and for increasing temporal resolution, e.g. in fMRI[70].

CAA research in Leiden

The significance of D-CAA for a greater scale, serving as a disease model for sCAA, has been instrumental in gaining awareness for D-CAA – a recognition that many rare or small-scale diseases often lack despite deserving it. This recognition of D-CAA is not only in the form of scientific advances, but also through inclusion as disease subtype in clinical trials which promotes faster and easier transition of effective and approved medication to D-CAA.

The proximity of the LUMC to the area where many people with D-CAA and potential D-CAA mutation carriers live (Katwijk and surroundings), has proven very helpful in studying D-CAA in the Leiden University Medical Center (LUMC). Therefore, from the eighties many studies with D-CAA as disease subtype have been performed at the LUMC. Studies investigating CAA that involve MRI and were performed from 2013 onwards are presented in Table 2[71-73]. During my PhD, I have been involved in the organization, data curation, scanning, and post-processing of MRI scans for all studies mentioned in Table 2, with exception of Clear-Brain and EDAN. Due to the ongoing status of Clear-Brain, I have only had a small contribution to scanning (technicalities) and post-processing involvement is anticipated. For EDAN, I have worked on project-specific post-processing for the study presented in Chapter 4.

Currently, a world-wide pharmaceutically sponsored randomized double-blind placebo-controlled study ('cAPPricorn'), is ongoing to test the effects and safety, tolerability, and pharmacodynamics of the drug ALN-APP. D-CAA is also included in this study as disease subtype.

Table 2. CAA related studies including MRI, performed at the LUMC from 2013 onwards

	Study name	Populations	Study period	Study visits	Study components	Additional notes
Placebo-controlled randomized trial	BATMAN	sCAA, n = 46 D-CAA, n = 12	2020 – 2024	2, with a 3-month interval	• 2 treatment arms: 1) placebo and 2) minocycline • 7T MRI • CSF from lumbar punctures • blood samples • cognitive tests • demographic and medical questionnaires	
Randomized pre-post trial	Clear-Brain	sCAA, n = 30 D-CAA, n = 30	Started in 2025 and is ongoing at moment of writing	3, with a 3-month interval	• 3 treatment arms: 1) LXB, 2) nVNS, and 3) LXB and nVNS • 7T MRI • CSF from lumbar punctures • blood samples • cognitive tests • demographic, medical, and sleep questionnaires	This study is ongoing at moment of writing, therefore the target inclusion numbers are provided.
Natural history studies	EDAN	D-CAA, n = 27 controls, n = 33	2013 – 2018	2, with a ~4-year interval	• 3 and 7T MRI • PiB-PET-CT scan • CSF from lumbar punctures • blood samples • cognitive tests • demographic and medical questionnaires	
	FOCAS(-light)	sCAA, n = 70 and subset of n = 34 for FOCAS-light	2018 – 2024	5, with 12-month intervals and a 24-month interval for the last visit	• 3 and 7T MRI • CSF from lumbar punctures • blood samples • cognitive tests • demographic and medical questionnaires • actigraphy sleep measurements • 24 hour and/or week blood pressure monitoring • EEG	FOCAS continued into FOCAS-light (single-visit version of FOCAS) from 2022 – 2024 to accommodate more inclusions.

	AURORA	D-CAA, n = 79 Controls, n = 5	2018 – 2024	5, with 12-month intervals and a 24-month interval for the last visit	Same measurements as FOCAS	
	TRACK (or AURORA -PLUS)	D-CAA, n = 49 Controls, n = 29 Unknown mutation status, n = 2	2021 – 2025 (until 2026 in Perth)	3, with 12-month intervals	Same measurements as FOCAS/AURORA, however: • including F18-Florbetaben [18F-FFB] PET-CT scan • no EEG • no sleep measurements • no blood pressure monitoring	Study is performed in both Leiden, the Netherlands, as in Perth, Australia. Inclusion numbers are from Leiden and Perth combined.
Cross-sectional observational study	BrightFocus	sCAA, n = 25 controls, n = 20	2022 – 2023	1	• 3T MRI with contrast agent • CSF from lumbar punctures • blood samples • cognitive tests • demographic and medical questionnaires	

Abbreviations. sCAA; sporadic Cerebral Amyloid Angiopathy, D-CAA: Dutch-type hereditary Cerebral Amyloid Angiopathy. 3/7T; 3/7 Tesla. MRI; magnetic resonance imaging. CSF; cerebrospinal fluid. LXB; low-sodium oxybate. nVNS; non-invasive vagus nerve stimulation. PiB; Pittsburgh compound B. PET-CT; positron emission tomography x-ray computed tomography. EEG; electroencephalogram.

Thesis outline

In this thesis, results from a number of studies on D-CAA and sCAA performed at the LUMC are described to increase our understanding of the mechanisms that contribute to disease progression, building upon the currently available literature. This thesis consists of three parts. In part I, chapters that cover CVR changes as measured with visually stimulated BOLD fMRI are presented. Starting with Chapter 2 in which the relationship between microstructural white matter integrity as measured through PSMD and CVR changes is assessed in D-CAA to increase understanding of mechanisms underlying the remote effects of Aβ accumulation. In Chapter 3, results from a one-year follow-up measure of CVR changes in both D-CAA and sCAA are presented in scientific abstract form, assessing the short-term changes. In part II, the focus lies on non-hemorrhagic brain injury in the form of enlarged PVS. PVS assessment in CAA has mainly been performed through qualitative analysis or limited quantification and the longitudinal progression of PVS remains largely unknown. Therefore, Chapter 4 covers cross-sectional and longitudinal quantification of PVS volume fraction in the total normal appearing white matter of the cerebrum in D-CAA. In part III, chapters are presented that are focused on hemorrhagic lesions, specifically CMBs, in both sCAA and D-CAA. Chapter 5 covers the distribution of CMBs along the cortical ribbon, focusing on the distribution between the sulcal and gyral part of the cortex, to assess hemorrhagic vulnerability along the cortex. Chapter 6 focuses on CMB clustering patterns within flow territories and interindividual clustering is assessed to increase mechanistic understanding of CMB occurrence. Finally, reports on acute CMBs have been scarce, therefore Chapter 7 covers the observation and occurrence of hyperintense CMBs on ultra-high field 7T MRI.