Staging cerebral amyloid angiopathy: from marker to model

Koemans, E.A.

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GENERAL DISCUSSION

1 General discussion

1. 1 Focus of this thesis

The main aim of this thesis was to investigate novel clinical and radiological markers for Cerebral Amyloid Angiopathy (CAA) to aid CAA diagnosis, increase our understanding of CAA pathophysiology and identify measures of disease severity and progression. This thesis was divided into three parts: **Part I** discussed (early) clinical symptoms and signs of CAA, **Part II** discussed MRI markers, and **Part III** combines the data from the previous chapters as well as data from literature to form a pathophysiologic framework for CAA progression, and tests this framework in participants with hereditary Dutch-type CAA (D-CAA).

1.2 Part I: Clinical symptoms and signs of CAA (Chapters 2 ,3 and 4)

The first part of this thesis focused on clinical markers of CAA. **Chapter 2** of this thesis showed that migraine with aura can be an inaugural symptom of D-CAA. As D-CAA is considered to be a model for sporadic CAA, albeit one with a more severe disease course and earlier onset, it seems logical to assume that migraine aura might also be found as an early symptom in patients with sporadic CAA, although this has not yet been systematically investigated. The finding that migraine with aura is a possible early sign of small vessel diseases such as CAA has clinical implications. It can help diagnosing CAA and can influence clinical decision making: long lasting single aura symptomatology in patients with CAA should be regarded as an alarm symptom which necessitates further physical and radiological examination. Due to the overlapping definitions of migraine aura and TFNE it is not possible to differentiate between these two phenomena, especially in the patients who do not suffer from (migraine) headache. Both TFNE and migraine (aura) are considered to be caused by cortical spreading depression (CSD). Therefore, we wonder if these two phenomena have the same underlying pathophysiological mechanism. Our findings could indicate that patients with CAA and other cerebral small vessel diseases are more susceptible to CSD, even in early phases of the disease. This higher susceptibility for CSDs was also found in a mouse model of CADASIL.[1] Other studies have suggested that this enhanced susceptibility is related to vascular dysfunction, a phenomenon found in early phases of both CADASIL and D-CAA.[2-4] We hypothesize that early microvascular damage of the intracerebral small vessels leads to vascular dysfunction which in turn causes increased susceptibility

for cortical spreading depression. Due to the overlapping definitions of migraine aura and TFNE, it is not possible to differentiate between these two phenomena, especially in the patients who do not suffer from (migraine) headache.

In **Chapter 3** we found that males seemed to have an earlier onset (sporadic CAA) and more severe hemorrhagic disease course (sporadic CAA and D-CAA) compared to females. In **Chapter 4** we investigated possible mechanisms for the sex-differences found in **Chapter 3**, and found an association of female sex with higher prevalence of parenchymal amyloid-β, but no sex-differences in prevalence of vascular amyloid-β (CAA), in a cohort of cognitively impaired patients. In a cohort of definite sporadic CAA patients, we found a higher microbleed but lower cSS proportion in females compared to males, and a higher local cortical iron burden in males. Exploratory analysis suggested a possible stronger negative relation between cortical CAA percentage area and iron density in males compared to females. Based on these results we conclude that the previously found sex differences in hemorrhage onset and progression in CAA progression are most likely not due to differences in global CAA severity between males and females. Other factors, such as vascular remodeling might contribute but a larger dataset is necessary to further investigate the complicated underlying mechanisms. In the past decades the number of studies investigating sex differences in medical research has grown exponentially. It has become increasingly clear that these differences exist on almost all levels, from social and behavioral to cellular and molecular levels.[5] The influence of sex in Alzheimer's disease is a widely researched topic, but no studies had previously been performed investigating sex differences in CAA. As we found that sex seems to influence CAA onset and disease course, this may provide new insights into CAA pathophysiology and novel avenues for treatment. The sex differences might partially be explained by environmental factors and comorbidities: males tend for instance to smoke more and have higher blood pressure (although this difference does not hold after menopause, and we corrected for the presence of hypertension and smoking in chapter 3), both factors which could influence vascular health.[6, 7] Another possible factor is hormonal status: it has been proposed that estrogen is protective against Alzheimer's disease dementia, that its depletion exacerbates Alzheimer's disease progress in post-menopausal females, and that the APOE gene is highly estrogen responsive.[8-10] The natural decrease in estrogen caused by the menopause has been found to be a risk factor for Alzheimer's disease: the menopause not only marks a neuroendocrine transition phase, but also induces a hypometabolic state associated with neurological symptoms.[11, 12] Previous studies have shown that this state is in turn associated with increased amyloid-β deposition.[11-14] A recent meta-analysis found that later menopausal age was associated with decreased risk of dementia, including Alzheimer's disease and vascular dementia.[15] Currently, several

studies have been published regarding the role of estrogen in Alzheimer's disease, including preliminary results of a double blind randomized trial investigating the effect of estrogen plus progesterone against placebo on atherosclerosis.[16-22] The studies, however, have conflicting results, and therefore the question if estrogen suppletion could be beneficial to Alzheimer's disease and CAA remains unanswered. Future studies should further investigate sex differences in CAA, including the role of menopause onset and hormone suppletion. If female hormones are indeed protective, then estrogen suppletion might be considered as a novel target for future CAA therapy trials.

1.3 Part II: Novel MRI markers for CAA (Chapters 5, 6, 7, 8 and 9)

The second part of this thesis investigates novel radiological markers of CAA. As MRI is the main diagnostic tool for CAA, novel MRI markers can help to diagnose CAA and to unravel the pathophysiology of this disease. In **Chapter 5** and **Chapter 6** we discovered two novel markers at ultra-high-field 7 Tesla MRI in patients with D-CAA and sporadic CAA: the striped occipital cortex and intragyral hemorrhages. A striped occipital cortex was defined as a pattern of separate, hypointense linear stripes at T_2*-weighted MRI, and intragyral hemorrhage was defined as parenchymal hemorrhage restricted to the juxtacortical white matter of an individual gyrus. The histopathology of the striped occipital cortex was investigated by our group in 2018. The pattern was found to be based on iron and calcium depositions of the penetrating cortical arteries in the occipital cortex.[23] Another study recently showed that the striped cortex is associated with occipital calcifications at CT, suggesting that the striped occipital cortex on MRI might represent an early stage of a more extensive process of calcification.[24] The pathophysiology of intragyral hemorrhages is less clear. It is striking that in these hemorrhages the juxtacortical white matter seems to be affected, as CAA predominantly affects cortical and leptomeningeal arterioles. In **Chapter 6** we found that all intragyral hemorrhages have cortical involvement, and that their shape on T2 TSE MRI seems to correspond to the shapes of enlarged perivascular spaces in the same regions. Based on this observation we hypothesize that intragyral hemorrhages are formed when cortical (micro) hemorrhages leak into the enlarged perivascular spaces, leading to their characteristic appearance on MRI. Prospective follow-up 7 Tesla data are necessary to test this hypothesis. The existence of the striped cortex and intragyral hemorrhages has clinical implications: their absence in deep perforator arteriopathy (DPA) related ICH seems to suggest specificity for CAA and therefore the markers could help diagnose CAA in case of doubt. In **Chapter 7** of this thesis we investigated CSF hyperintensities at non-contrast

7 Tesla FLAIR in CAA. We hypothesize that the found CSF hyperintensities are a sign of (subtle) leakage of plasma products such as protein and hemosiderin, which leads to CSF T1-shortening at 7 Tesla FLAIR. Previous studies have already suggested that CAA can cause blood-brain barrier (BBB) disruption, and our findings support this hypothesis.[25] This subtle BBB leakage could also play a role in the pathophysiology of cortical superficial siderosis (cSS): the pathophysiology of cSS is not completely understood and one hypothesis is that it is caused by continuous leakage of blood-products from vessels damaged by CAA.[26] It could be hypothesized that the CSF hyperintensities that we discovered are an early indicator for future cSS, however this hypothesis can't be proved based on our current data. The CSF hyperintensities are of interest for CAA research: the dynamic properties of the marker suggest potential to be used to monitor disease activity. That the marker is best identifiable at 7T MRI makes it less suitable for implementation into clinical practice, although CSF hyperintensities could be identified at 3T in retrospect. The current study is only a first explorative effort into investigating the properties of this marker. Future studies are necessary to further investigate the abilities of CSF hyperintensities at 7 Tesla FLAIR as a possible dynamic marker of BBB leakage in CAA, to formulate criteria for identifying the marker at 3T MRI and to investigate its prognostic properties.

Results of **Chapter 5**, **Chapter 6** and **Chapter 7** demonstrate the advantage of 7 Tesla MRI; the more detailed examination of the brain parenchyma leads to the discovery of novel markers and new theories regarding CAA pathophysiology. However, the use of 7 Tesla MRI also has disadvantages. 7 Tesla MRI is only available in a few centers around the world and is therefore not used in a clinical setting. Translation of novel findings to field strengths more commonly used in clinical practice is a challenge. Many of the radiological imaging criteria used in clinical practice (such as the Boston criteria) have not been validated for 7 Tesla MRI. Furthermore, the 7 Tesla MRI scan is taxing for participants due to the long scan duration. This creates a selection bias as patients who are not able to lie still for prolonged periods of time, such as those with severe clinical symptoms due to CAA, cannot undergo 7 Tesla MRI. Several sequences at 7T MRI, including the T2*-weighted sequence, are more sensitive to motion and image quality often suffers substantially from motion artifacts when patients become - even slightly - restless during scanning procedures. Efforts to decrease scan duration will inevitably be at the cost of other important scan parameters, and frequently induce artifacts that hinder image analysis.

In **Chapter 8** and **Chapter 9** we used 3 Tesla MRI to investigate novel markers for D-CAA and sporadic CAA: cerebellar superficial siderosis (SS) and mesobleeds. We consider cerebellar SS in CAA (**Chapter 8**) to be different from infratentorial

superficial siderosis (iSS), a phenomenon described previously in literature.[27] The presence of cerebellar SS in patients with CAA supports recent insights that the superficial cerebellum is also affected by CAA, probably via the same pathophysiological mechanisms as the supratentorial lobar regions of the brain. Not all patients with cerebellar SS also had supratentorial cSS or lobar ICH; therefore the finding of cerebellar SS could strengthen CAA diagnosis and might even improve the Boston criteria. Cerebellar SS was not present in presymptomatic patients and therefore it seems to be a late marker of the disease, similar to supratentorial cSS. Further studies are necessary to determine whether cerebellar SS has the same prognostic value for ICH prediction as supratentorial cSS. In **Chapter 9** of this thesis we investigate hemorrhage distribution in patients with sporadic CAA and D-CAA. We especially focus on the presence of hemorrhages with the characteristics of a macrobleed (hemorrhages shaped irregularly and/or containing a cystic cavity) smaller than 10 mm, which we called mesobleeds. We found that mesobleeds are abundantly present in patients with D-CAA and sporadic CAA and have a different diameter and volume distribution compared to micro- and macrobleeds, which challenges previous research regarding the size of macro- and microhemorrhages. The results of **Chapter 9** illustrate that imaging rating criteria should be continuously updated in order to implement improvements in imaging techniques.

1.4 Part III: Pathophysiologic Framework for CAA progression (Chapters 10 and 11)

In **Chapter 10** of this thesis we the literature on CAA related biomarkers and their proposed temporal ordering to formulate a framework and timeline for CAA progression. Finally, in **Chapter 11**, we investigate the temporal ordering of biomarkers in participants with presymptomatic and symptomatic D-CAA to exploring if this is in line with the earlier formulated framework. The initial idea for the formulation of such a framework for CAA pathophysiology came from a previously published, similar framework for Alzheimer's disease, proposed by Clifford Jack and colleagues in 2010 (and since then updated to incorporate novel evidence).[28, 29] The framework in this thesis is data driven, and is based on evidence from in and ex-vivo studies in humans as well as in transgenic mouse models. Via this framework we aimed to illustrate the current theories regarding the CAA disease process, from earliest subclinical stages to clinically manifest disease. We hope that the framework will be modified in future as the field further progresses.

It has been speculated before that the range in CAA disease onset and course is proof for the influence of unknown (epi)genetic or environmental factors. It has

even been suggested that there are different possible CAA phenotypes.[30] Another purpose of the pathophysiological framework is to visualize the effect of modifying factors for CAA on progression: different factors can influence CAA in different ways, as demonstrated below using a modified version of Figure 1 from **Chapter 10** and figure 3 from **Chapter 11**. Figure 1B demonstrates effects of possible factors influencing amyloid-β deposition onset and rate. These factors could influence amyloid-β production or type (such as the D-CAA mutation in the APP gene or APOE genotype), or overall cerebrovascular clearance (such as past head trauma or brain surgery). Figure 1C demonstrates effects of possible factors influencing sensitivity to accumulated amyloid-β. Examples of such are sex (with female sex being a protective factor, as demonstrated in this thesis), factors that influence cerebral inflammation, or factors affecting overall vascular health (such as hypertension and smoking). There are several biomarkers possibly associated with CAA that were not included in the framework, as we consider either their temporal ordering or specificity for CAA to be yet unclear. These markers include for example blood-brain barrier permeability and markers associated with inflammation and neurodegeneration such as tumor necrosis factor (TNF) and Glial Fibrillary Acidic Protein (GFAP) in plasma and CSF.[25, 31] Of some physiological processes, such as brain clearance via glymphatic or perivascular pathways, it is hypothesized that they play an important role in the pathophysiology of CAA, but no reliable way has yet been found to correctly measure them.[32] As these markers represent processes most likely occurring early in the disease cascade, it is probable that once they can be reliably measured, their curves could be added to the left (presymptomatic) side of the hypothetical model; occurring after amyloid-β accumulation but possibly prior to non-hemorrhagic injury. Another problem in tracking CAA disease course is that some of the markers, including CSO-EPVS, WMH and cSS, are usually scored via categorization. Especially in symptomatic patients this causes problems, as these patients are often already measured in the highest category of the marker at baseline and therefore measuring progression is not possible with the current methods. A possible solution to this is to start quantifying these markers, which will increase sensitivity to disease progression.[33]

Figure 1: Hypothetical schematic model for CAA progression and influencing factors.

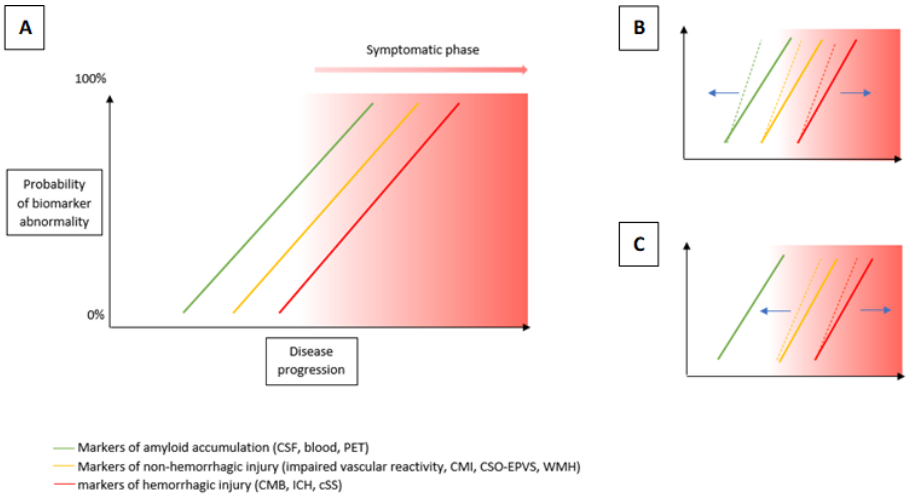

Figure 1A shows a modified version of figure 1 from **Chapter 10** *of this thesis, demonstrating a hypothetical schematic model for CAA disease progression, based on three different groups of biomarkers: markers of* amyloid-β *accumulation, (MRI) markers of non-hemorrhagic injury and (MRI) markers of hemorrhagic injury. The hypothetical temporal ordering of the biomarker groups is discussed in more detail in Chapter 10. Figure 1B illustrates hypothetical factors influencing onset (blue arrows) or rate (dotted lines) of* amyloid-β *deposition. Figure 1C illustrates hypothetical factors influencing sensitivity to accumulated* amyloid-β *(blue arrows and dotted lines).*

1.5 Methodological considerations

The various chapters in this thesis had different designs. In **Chapter 2** and part of **Chapter 3** we used a retrospective database with clinical and radiological routine care data of patients with D-CAA. With these type of retrospective databases, data collected for clinical care can be used for research. Especially in cohorts of rare diseases such as D-CAA this is a useful and essential tool. The major disadvantage of these data is that they are not collected using structural questionnaires and are therefore subjected to (recall) bias. We consider this form of research to be suitable for initial, exploratory studies, which should be followed by a secondary prospective study to confirm the findings. In **Chapters 4, 5, 6, 7, 8** and **9** as well as in part of **Chapter 3** we used data from several different cohorts containing data collected using a prospective study design. The main advantage of this type of data is that they were acquired in a structural way for all participants within the same cohort, which may limit recall bias, may enables a more reliable comparison between patients as all data was collected at the same day via the same method. An important limitation of a prospective cohort design is that it creates a selection

bias regarding disease severity, as the most affected patients are more often not able or willing to participate in prospective studies. In each chapter of this thesis we carefully discussed whether cohorts could be compared, and assessed possible sources of confounding prior to analysis to adjust for this.

Because D-CAA is a relatively rare genetic disease, the sample size in our studies is often relatively low. This can lead to statistical difficulties; for example in **Chapter 5** where the sample sizes of the groups were too small for statistical analysis. However, there are several reasons why the advantages of investigating D-CAA outweigh this disadvantage. Pathologically, sporadic CAA and D-CAA are similar, although D-CAA has an earlier onset and more severe disease course.[34] As D-CAA is a genetic disease patients can be tested for the causal mutation decades before the disease becomes symptomatic which allows for investigation of the presymptomatic phase in patients with a 'certain' diagnosis of CAA during life, instead of a 'possible' or 'probable' diagnosis which is usually the case in patients with sporadic CAA. Furthermore, patients with D-CAA have a relatively 'pure' form of CAA; they are younger at onset of the disease than patients with sporadic CAA and therefore suffer from less age-related comorbidities such as hypertension and hypercholesterolemia. In patients with sporadic CAA, such age-related comorbidities do play a role. In **Chapter 5** we found that 36% of the patients initially diagnosed with probable sporadic CAA according to the modified Boston criteria based on clinical 1.5 or 3 Tesla MRI had deep micro or macro hemorrhages on ultra-high-field 7 Tesla MRI, changing their diagnosis from sporadic CAA to a mixed form of CAA and DPA. It is current practice to consider small vessel disease as a spectrum with CAA and DPA on opposing ends. Many patients with sporadic CAA will have signs of DPA, or might develop these changes over time as they are strongly related to age and hypertensive arteriopathy. In CAA research, the in or exclusion of 'mixed-type' small vessel disease patients forms a dilemma. Recent studies have shown that the majority of mixed type small vessel disease is probably caused by DPA in the absence of CAA.[35] However, in our clinical practice we often encounter patients with predominantly CAA related MRI markers and only limited DPA; these patients are often diagnosed as 'pure' CAA based on clinical symptoms and radiological features such as lobar ICH or cSS, and only after perusal of their MRI scans signs of DPA is found. In patients with predominantly CAA related MRI markers, such as cSS and lobar ICH, CAA is more likely to be present, and these are the patients which we encountered in **Chapter 5**.[36] In our opinion, cerebral small vessel disease is a spectrum, and future research should concentrate – in addition to the distribution - on the ratio of the different MRI markers in deep

and lobar locations within patients in order to determine whether CAA or DPA is more dominant. The Boston criteria have been validated for pure CAA only, as have most clinical guidelines, and therefore it is of the utmost importance to keep investigating the mixed type patient group. We therefore recommend to include these patients in future studies as we think it is important to keep investigating this group, both together with and separate from pure sporadic CAA.

1.6 Future perspective

1.6.1 Natural history studies

CAA research has progressed impressively over the past decades, bringing us closer and closer towards methods for prevention and treatment. However, there are some key problems that need to be solved to further 'unravel' CAA pathophysiology. First, although there are many hypothetical approaches for preventing or treating amyloid-β accumulation in the cerebral vessels (for example by increasing clearance or inhibiting production of the peptide) it is yet unclear why amyloid-β, a protein produced in the brain of all humans, causes CAA in some patients and not in others, nor why the disease course is so variable.[37] One branch of CAA research focusses on why CAA occurs, using animal models as well as revolutionary 'vessel on a chip' models to closely investigate amyloid-β deposition and clearance pathways in CAA and Alzheimer's disease.[38, 39] In mouse models of CAA, methods have been developed to visualize CAA affected vessels in vivo using in vivo two-photon microscopy.[40] These models enable the study of amyloid-β accumulation during life in all disease phases, as well as the effect of any possible preventive measures or treatments.[40] Although, of course, there is a limit to the parallel between mice and men. Next to ex-vivo studies and animal models, natural history studies in humans are used to investigate the natural disease course in presymptomatic and symptomatic patients with hereditary and sporadic CAA, as well as factors that influence disease variability and onset. Two such studies have been set up in the LUMC in 2018, FOCAS and AURORA, and this thesis has used the first data from these studies to uncover novel biomarkers for CAA and to investigate sex as a possible disease influencing factor. Future follow-up data of these studies will shed further light on onset, long-term disease course and risk factors for CAA. A last key problem in CAA research is the lack of reliable biomarkers to track disease progression over a short period of time and to measure the effect of a possible treatment. Possible candidate markers are not only radiological, including PET and fMRI, but also blood, CSF and clinical markers, such as migraine aura as described

in this thesis. Investigating novel markers and their progression, as is done in this thesis, adds to the spectrum of CAA. Especially dynamic factors, such as the CSF hyperintensities described in **Chapter 8**, could be interesting candidates for monitoring disease progression. Another promising dynamic marker could be the measurement of brain (amyloid-β) clearance. Several novel MRI techniques show promising methods for measuring in vivo brain clearance in humans, and future studies should investigate the prognostic value of these measurements in patients with CAA.[41, 42] Further investigation of follow-up data from AURORA and FOCAS will play an important role in uncovering which markers are most reliable in monitoring disease progression and outcome prediction.

1.6.2 CAA treatment trials

In the search for a treatment for CAA one could aim at different stages in the disease. In the earliest known phases, modification amyloid-β accumulation itself is the main target. A possible method for targeting amyloid-β could be DNA or RNA modifying therapies (exon skipping), or messenger RNA or RNA interference therapeutics.[43-45] DNA and RNA therapies for CAA are currently still mostly investigated in animal models, although recently a Phase 1 trial investigating RNA interference therapeutics targeting the amyloid precursor protein has been started in humans with Alzheimer's disease (*https://clinicaltrials.gov/ct2/show/NCT05231785*). Another possible method for targeting amyloid-β is to use monoclonal antibodies against amyloid-β or APOE, thereby decreasing vascular amyloid-β load and improving vascular function. Previous studies have shown that some of these antibodies show promising results, and recently one of these has even been approved for clinical use in Alzheimer's disease, targeting both vascular (CAA) and parenchymal amyloid-β.[46, 47 48, 49] However, a large group of experts remains critical of the use of these drugs for CAA, as so far none of the trials has shown positive results regarding clinical outcome. Furthermore, the use of these antibodies is not without risk, as they can cause severe CAA-like inflammatory or hemorrhagic manifestations.[50-53] It is thought that especially the removal of vascular amyloid-β causes hemorrhaging: in a severe stage of CAA the smooth muscle cells of the vessel have been completely destroyed and replaced by amyloid-β, and vascular remodelling occurs. If the vascular amyloid-β is then removed, the vessel loses all stability, causing it to rupture. Although monoclonal antibody therapy against amyloid-β could be promising, the unfortunate reality is that the current Food and Drug Administration (but not European Medicines Agency) approved, expensive therapies have dangerous side-effects, without the clinical benefits outweighing

these costs.[51, 52, 54] Due to these severe complications it would be more ideal to treat CAA in an earlier stage, either by preventing accumulation of amyloid-β or by removing vascular amyloid-β before smooth muscle cell degeneration. A lot of interest has also been generated for increasing amyloid-β clearance, for example by modification of perivascular or glymphatic clearance.[55] One possible interesting way of doing this is by improving sleep: there is evidence that sleep plays an important role in amyloid-β clearance, and lack of sleep or sleep deprivation can increase amyloid-β burden.[56] Another interesting pathway would be to increase clearance by increasing vasomotion through visual stimulus, a method which has shown promising results in mice models.[38, 57]

Next to finding possible ways of treating the early phases of CAA, another branch of CAA research focusses on modifying the disease in a later stage. A possible approach for this is to target CAA related inflammation. Next to the clinical phenotype of inflammatory CAA, CAA-ri, it is known that inflammation is present in CAA. An ongoing hypothesis is that CAA triggers angiogenesis as a reaction to this inflammation, with the new-formed vessels being susceptible to leakage.[58-60] Pre-clinical studies which attempted to treat this inflammation in animal models of CAA by treating with the antibiotic minocycline showed promising results; the drug prevented ICH occurrence and improved behavioral outcome, as well as reducing gliosis and expression of inflammatory genes and gelatinases.[61-63] To investigate whether minocycline influences CAA in humans, the LUMC started a randomized placebo controlled treatment trial in 2020, investigating minocycline in patients with hereditary and sporadic CAA (the BATMAN study). This study will not only lead to new insights into disease pathophysiology and possible treatment options for CAA, it is also an important milestone in CAA research in general, as it is only the second treatment trial in CAA, and the first that includes patients with D-CAA.

1.6.3 Recommendations

For the future of CAA research it is of the utmost importance that natural history studies which include presymptomatic patients as well as patients with mixed type cerebral small vessel disease are continued, to learn more about this variable disease in all its stages, as well as within the clinical spectrum of cerebral small vessel disease. Via (international) collaborations, larger prospective datasets should be set up to better investigate CAA risk factors and disease modifying factors such as sex, menopause and hormone suppletion. A better understanding of CAA pathophysiology and amyloid-β clearance mechanisms is an absolute necessity for the generation of future therapies. A major step towards understanding amyloid-β

accumulation is the use of in vivo two-photon microscopy in live CAA mouse models to visualize affected blood vessels in the brain.[40] Other important steps for CAA disease management contain a focus on improving general vascular health by managing lifestyle and cardiovascular risk factors such as hypertension, smoking and hypercholesterolemia. Clinical follow up of CAA patients should always include strict blood pressure management, as this is so far the only manageable risk factor for CAA.[64] Lastly, clinical care of patients with CAA needs to focus on after stroke and dementia care, including recovery and rehabilitation as well as more attention for psychological and psychiatric symptoms, which frequently occur in these populations.[65] Last, novel discoveries in CAA should always be placed in context of what is known or considered true regarding the pathophysiology; we formulated a framework for CAA pathophysiology in **Chapter 10**, and will continue to modify this framework according to future discoveries.

1.7 Concluding remarks

In conclusion, this thesis describes novel clinical and radiological biomarkers for CAA and combines knowledge of CAA-related biomarkers into a pathophysiologic framework. CAA is characterized by a complex disease cascade and a highly variable, and therefore difficult to predict, disease course. However, it is just this variability that gives hope for the future: by investigating the driving forces behind this variability we can find methods of modifying disease course and possible ways of prevention and treatment, and in vivo biomarkers play a vital role in this search.

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