Microvascular contributions to dementia

Citation for published version (APA):

Freeze, W. M. (2018). *Microvascular contributions to dementia: exploring the role of blood-brain barrier leakage in cerebral small vessel disease and Alzheimer disease*. [Doctoral Thesis, Maastricht University]. Ridderprint BV. <https://doi.org/10.26481/dis.20180628wf>

Document status and date:

Published: 01/01/2018

DOI:

[10.26481/dis.20180628wf](https://doi.org/10.26481/dis.20180628wf)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Microvascular contributions to dementia

Exploring the role of blood-brain barrier leakage in cerebral small vessel disease and Alzheimer disease

© Whitney M. Freeze, Maastricht 2018

All rights reserved. No part of this book may be reproduced or transmitted in any form or by any means, without prior permission in writing by the author, or when appropriate, by the publishers of the publication.

Cover by Evelien Jagtman

Layout by Whitney Freeze

Printing: Ridderprint BV, Ridderkerk

ISBN: 978-94-6299-986-2

Microvascular contributions to dementia

Exploring the role of blood-brain barrier leakage in cerebral small vessel disease and Alzheimer disease

PROEFSCHRIFT

Ter verkrijging van de graad van doctor aan de Universiteit Maastricht, op gezag van de Rector Magnificus, Prof. dr. Rianne M. Letschert, volgens het besluit van het College van Decanen, in het openbaar te verdedigen op donderdag 28 juni 2018 om 14.00 uur

door

Whitney Marilyn Freeze

Geboren op 25 Mei 1990 te Leiderdorp

Promotoren

Prof. dr. F.R.J. Verhey

Prof. dr. ir. W.H. Backes

Co-promotor

Dr. H.I.L. Jacobs

Beoordelingscommissie

Prof. dr. P.A.M. Hofman, voorzitter

Prof. dr. A.P. Aldenkamp

Prof. dr. G.J. Biessels (UMC Utrecht)

Prof. dr. J. Hendrikse (UMC Utrecht)

Prof. dr. D.J. Linden

The research described in this thesis was performed at the School for Mental Health and Neuroscience, Department of Psychiatry&Neuropsychology and Department of Radiology, Maastricht University, the Netherlands; and at the Department of Neurology and J. Philip Kistler Stroke Research Group, Massachusetts General Hospital, Harvard Medical School, Boston, USA; and was financially supported by Alzheimer Nederland and Stichting 2Bike4Alzheimer.

Printing of this thesis was kindly supported by Alzheimer Nederland (Amersfoort) and Maastricht University. In addition, financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged.

Maastricht University

Funded by

Hartstichting

CONTENTS

CHAPTER 1 **7**

GENERAL INTRODUCTION, THESIS AIM AND OUTLINE.

CHAPTER 2 **17**

WHITE MATTER HYPERINTENSITIES POTENTIATE HIPPOCAMPAL VOLUME REDUCTION IN NON-DEMENTED OLDER INDIVIDUALS WITH ABNORMAL AMYLOID-BETA.

Journal of Alzheimer's disease, 2017

CHAPTER 3 **37**

ON THE LINK BETWEEN BLOOD-BRAIN BARRIER LEAKAGE, WHITE MATTER HYPERINTENSITIES, NEURODEGENERATION, AND COGNITION.

Submitted

CHAPTER 4 **73**

PERICORTICAL ENHANCEMENT ON DELAYED POSTGADOLINIUM FLUID-ATTENUATED INVERSION RECOVERY IMAGES IN NORMAL AGING, MILD COGNITIVE IMPAIRMENT, AND ALZHEIMER DISEASE.

American Journal of Neuroradiology, 2017

CHAPTER 5 **91**

BLOOD-BRAIN BARRIER DYSFUNCTION IN SMALL VESSEL DISEASE RELATED INTRACEREBRAL HEMORRHAGE - A SYSTEMATIC REVIEW.

Submitted

CHAPTER 6 **131**

BLOOD-BRAIN BARRIER LEAKAGE AND MICROVASCULAR LESIONS IN CEREBRAL AMYLOID ANGIOPATHY: A POST MORTEM MRI-HISTOPATHOLOGY STUDY.

CHAPTER 7 **153**

GENERAL DISCUSSION.

ADDENDUM

SUMMARY	170
SAMENVATTING	172
KNOWLEDGE VALORIZATION	174
DANKWOORD	179
CURRICULUM VITAE	185
PUBLICATIONS	187
THESIS DEFENCES FROM MHEENS	188

CHAPTER 1

GENERAL INTRODUCTION, THESIS AIM AND OUTLINE.

Late-onset Alzheimer disease (AD) is a neurodegenerative disease that is characterized by progressive cognitive and functional decline ¹. Although AD is believed to be the most common cause of dementia, the majority of AD autopsy cases show at least one additional type of pathology, which is of vascular origin in most cases ²⁻⁶. *In vivo* studies have confirmed that cerebrovascular disease is prevalent in AD ⁷, and that both types of pathology share many risk factors ⁸⁻¹⁰. Moreover, recent studies have established that both types of pathology are associated with cerebral atrophy and cognitive decline ¹¹⁻¹³. Despite these significant insights, cerebrovascular pathology remains frequently ignored in research disease models and clinical criteria for AD. For example, cerebrovascular damage is not directly included in a recently developed classification system for AD biomarkers ^{14, 15}, and evidence of substantial cerebrovascular damage is even an exclusion criterion for probable AD-type dementia diagnosis ¹⁶. Recognition of the multifactorial biological underpinnings of dementia and research into cerebrovascular dysfunction from disease onset onwards form crucial steps towards our understanding of the pathophysiological mechanisms underlying cognitive decline, and could possibly contribute to the development of effective, disease-modifying therapies ¹⁷. Therefore, the general aim of this thesis is to examine the role of cerebrovascular abnormalities in relation to cognitive impairment and AD.

The multifactorial etiology of Alzheimer disease

According to the National Institute on Aging-Alzheimer's Association criteria, AD forms a continuum that can be divided into three stages: preclinical AD, mild cognitive impairment (MCI) due to AD, and dementia due to AD ^{16, 18, 19}. AD is pathologically characterized by cerebral accumulation of the amyloid- β peptide in the form of extracellular senile plaques, and intracellular deposition of hyperphosphorylated tau protein as neurofibrillary tangles. These pathological processes start many years before clinical symptoms become evident, and are followed by significant neuronal loss and synaptic dysfunction, and eventual cognitive decline. Despite significant advances in our understanding of disease mechanisms and course ¹⁵, the cause of the abnormal protein accumulation in AD remains unknown, and no treatments are available to slow down or stop the disease process. This lack of knowledge can be explained by the long preclinical phase and complex, multifactorial etiology of late-onset AD ²⁰. Improving our understanding of vascular contributions to AD may improve our understanding of how AD leads to cognitive decline.

Vascular damage in AD: The blood-brain barrier and cerebral small vessel disease

The cerebral vasculature provides essential nutrients and oxygen to all types of brain cells and is thereby of vital importance to brain health. Cerebral blood vessels have unique structural and functional properties that distinguish them from vessels within other organs. One of the most distinctive features of the cerebral vessels is the blood-brain barrier (BBB)²¹. The BBB is formed by an interactive cellular complex of endothelial cells that form the inner wall of the cerebral blood vessels, and surrounding mural cells and glial cells (figure 1)²²⁻²⁴. The endothelial cells are separated by tight junctions that restrict molecular exchange between the blood and the surrounding brain tissue²⁵. One of the key functions of the BBB is to maintain cerebral homeostasis by regulating selective transport between the blood and brain. This transport is bidirectional: essential nutrients and energy metabolites are transported into the brain, and metabolic waste products can be removed from the brain via interstitial fluid pathways into the blood²⁵. In addition, the BBB protects of the neuronal environment by excluding neurotoxic plasma components and minimizing inflammation²⁶. Vascular dysfunction in the form of BBB leakage of plasma components can have detrimental effects on brain health²⁷. Interestingly, several studies point towards a crucial role of BBB dysfunction in the pathogenesis and progression of AD, and dysfunction of the BBB forms an important link between AD and cerebrovascular pathology^{23, 28-33}.

Figure 1: Schematic cross-sectional representation of the interactive cellular blood-brain barrier (BBB) complex at the level of a capillary blood vessel (reproduced from Zenaro et al.)²⁴.

BBB dysfunction is considered to be the core mechanism underlying cerebral small vessel disease (cSVD)^{34, 35}, the most common cause of cerebrovascular pathology in AD³⁶. The term

cSVD encompasses all the pathological processes that affect the small vessels within the brain, including small arteries, arterioles, capillaries and small veins ³⁷. The two most common types of cSVD include hypertensive vasculopathy (HV) and cerebral amyloid angiopathy (CAA) ³⁷. In HV, loss of smooth muscle cells, luminal narrowing, and thickening of the vessel wall affect the deep penetrating small vessels in the brain ³⁸. Cerebral amyloid angiopathy is characterized by progressive cortical and meningeal deposition of the amyloid- β protein within the walls of small arteries, arterioles, and sometimes capillaries ³⁹. Both types cSVD are associated with the development of (micro)vascular lesions, including cerebral microinfarcts, lacunar infarcts, white matter abnormalities, and cerebral microbleeds, and with an increased risk of ischemic and hemorrhagic stroke and cognitive impairment ⁴⁰⁻⁴³.

Post mortem and in vivo imaging of blood-brain barrier leakage in humans

Measuring BBB dysfunction in AD has been a topic of interest over the past thirty years ⁴⁴. Dysfunction of the BBB can be measured as plasma protein leakage into the cerebral vasculature and parenchyma with immunohistochemical techniques. However, because the leakage of plasma components is only subtle in AD, and subtle BBB dysfunction is also associated with normal aging and other neurological disorders associated with aging ^{30, 44}, neuropathological studies are inconclusive as to whether plasma protein leakage is increased in AD compared with normal aging ⁴⁵⁻⁵¹. A significant strength of histopathological examination of BBB leakage, is the high spatial resolution of microscopy, which makes it possible to study pathological changes at the level of individual small vessels and correlate these to other neuropathological markers of AD. This is particularly interesting with regard to pathological AD correlates for which there are currently no means available to measure their severity *in vivo*, such as CAA ⁵².

Others have attempted to measure BBB dysfunction with magnetic resonance imaging (MRI). An important advantage of MRI over histopathology is the relatively non-invasive nature of the technique, which enables studying both structural and functional properties of the brain *in vivo* and repeatedly. Another advantage of MRI is that it provides three-dimensional information on the anatomical location of BBB leakage, with coverage of almost the entire brain, while histopathological studies typically have to rely on a selection of small brain samples. BBB dysfunction can be measured with T1-weighted dynamic contrast enhanced (DCE)-MRI in the form of leakage of a gadolinium-based contrast agent from the blood into the cerebrospinal fluid and brain parenchyma ⁵³. Recent studies suggest that DCE-MRI is sensitive enough to detect increased BBB leakage in (early) AD and cSVD compared with cognitively normal age-matched

controls⁵⁴⁻⁵⁶. Because MRI can concurrently capture the full profile of cSVD hallmarks and typical AD neurodegenerative processes such as hippocampal atrophy, MRI is a valuable method in assessing the complex relationship between these markers and blood brain barrier leakage.

Gaps in the current literature

It remains unclear how BBB dysfunction and cSVD contribute to neurodegeneration, cerebrovascular lesions, and clinical symptoms in AD. With the availability of increasingly sensitive techniques such as DCE-MRI that allow localization and quantification of BBB leakage in humans and *in vivo*, it is now feasible to bridge the gap between well-established biomarkers of cerebrovascular pathology, neurodegeneration and cognitive decline on the one hand, and emerging markers of BBB leakage on the other hand. Besides the application of DCE-MRI, the utility of other available MRI techniques aimed at measuring leakage of gadolinium-based contrast agents should be explored. Such techniques may provide complementary information about BBB leakage and may require shorter scan acquisition times. For example, post-contrast fluid-attenuated inversion recovery (FLAIR) imaging has already proven useful in measuring BBB leakage in other neurological diseases, but has not yet been evaluated in AD⁵⁷⁻⁵⁹. Because CAA and HV are prevalent in AD and strongly associated with cerebrovascular lesions, it is tempting to assume that BBB dysfunction may contribute to cognitive decline by playing a role in the etiology of these lesions, but studies on this topic are scarce. Filling these gaps will increase our understanding of microvascular contributions to dementia, which is important for the improvement of prognostic accuracy and to facilitate the development of future treatment strategies aimed at delaying cerebrovascular lesion development and cognitive decline.

Thesis aim and outline:

The general aim of this thesis was to investigate whether microvascular dysfunction in the form of BBB leakage is associated with cerebrovascular lesions and common markers of AD pathology, and how these markers are associated with cognitive performance in memory clinic patients and cognitively normal older individuals. The research described in the following chapters is based on the following research questions.

Can amyloid- β pathology and cSVD interact in their relationship with hippocampal volume?

Although the association between cSVD and AD pathology is well established^{60, 61}, it remains unclear whether both types of pathology interact. In **chapter 2** we assessed the association between

MRI-visible cSVD severity and hippocampal volume in memory clinic patients with normal and abnormal amyloid- β status using data from the Dutch multicenter LeARN study.

Is (local) BBB leakage associated with cSVD severity, hippocampal volume, and cognitive performance?

In **chapter 3** we examined BBB leakage measured with DCE-MRI in predefined regions (gray matter, white matter, white matter hyperintensities, hippocampus) in cognitively normal individuals and memory clinic patients from Maastricht University Medical Center. We assessed the associations between regional BBB dysfunction, cSVD severity, hippocampal volume, and cognitive performance.

Can we measure BBB leakage with post-contrast FLAIR imaging in older individuals with normal cognition and in memory clinic patients?

Chapter 4 describes how a relatively understudied MRI technique, post-contrast FLAIR imaging, can be used to measure focal pericortical vascular leaks in cognitively normal older individuals and in memory clinic patients from Maastricht University Medical Center. We also examined the association between presence of this form of BBB dysfunction and other MRI markers of cerebrovascular damage, and how this neuroradiological phenomenon develops over a period of two years.

Is BBB dysfunction associated with cSVD-related hemorrhagic brain lesions?

Chapter 5 constitutes a systematic review that summarizes the literature on BBB dysfunction in the etiology of cSVD related spontaneous intracerebral hemorrhage and cerebral microbleeds. Both animal and human studies were included in order to capture possible causal mechanisms (longitudinal animal studies) and evidence in human *post mortem* and *in vivo* studies.

Is BBB leakage associated with CAA severity and microvascular lesions?

In **chapter 6**, we examined how BBB dysfunction is related to CAA severity and microvascular lesions on a histopathological level. We used *post mortem* tissue that was donated to the J. Philip Kistler Stroke Research group at Massachusetts General Hospital (Boston, USA). Six CAA patients and two non-neurological aged individuals without evidence of CAA pathology in the brain were included in this study. Local CAA severity and BBB leakage were related to the number of microvascular lesions detected on *post mortem* MRI.

REFERENCES

1. Barker WW, Luis CA, Kashuba A, et al. Relative frequencies of Alzheimer disease, Lewy body, vascular and frontotemporal dementia, and hippocampal sclerosis in the State of Florida Brain Bank. *Alzheimer disease and associated disorders* 2002;16:203-212.
2. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* 2007;69:2197-2204.
3. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. *Jama* 1997;277:813-817.
4. Attems J, Jellinger KA. The overlap between vascular disease and Alzheimer's disease -lessons from pathology. *BMC medicine* 2014;12:206.
5. Wilson RS, Segawa E, Boyle PA, Anagnos SE, Hizez LP, Bennett DA. The natural history of cognitive decline in Alzheimer's disease. *Psychology and aging* 2012;27:1008-1017.
6. Stephan BC, Matthews FE, Ma B, et al. Alzheimer and vascular neuropathological changes associated with different cognitive States in a non-demented sample. *Journal of Alzheimer's disease : JAD* 2012;29:309-318.
7. Kester MI, Goos JD, Teunissen CE, et al. Associations between cerebral small-vessel disease and Alzheimer disease pathology as measured by cerebrospinal fluid biomarkers. *JAMA neurology* 2014;71:855-862.
8. Kivipelto M, Helkala EL, Laakso MP, et al. Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *BMJ (Clinical research ed)* 2001;322:1447-1451.
9. Skoog I, Lernfelt B, Landahl S, et al. 15-year longitudinal study of blood pressure and dementia. *Lancet* 1996;347:1141-1145.
10. Tan ZS, Seshadri S, Beiser A, et al. Plasma total cholesterol level as a risk factor for Alzheimer disease: the Framingham Study. *Archives of internal medicine* 2003;163:1053-1057.
11. Vemuri P, Knopman DS. The role of cerebrovascular disease when there is concomitant Alzheimer disease. *Biochimica et biophysica acta* 2015.
12. Guzman VA, Carmichael OT, Schwarz C, Tosto G, Zimmerman ME, Brickman AM. White matter hyperintensities and amyloid are independently associated with entorhinal cortex volume among individuals with mild cognitive impairment. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2013;9:S124-131.
13. Park JH, Seo SW, Kim C, et al. Effects of cerebrovascular disease and amyloid beta burden on cognition in subjects with subcortical vascular cognitive impairment. *Neurobiology of aging* 2014;35:254-260.
14. Jack CR, Jr., Bennett DA, Blennow K, et al. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* 2016;87:539-547.
15. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *The Lancet Neurology* 2013;12:207-216.
16. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2011;7:263-269.
17. Drachman DA. The amyloid hypothesis, time to move on: Amyloid is the downstream result, not cause, of Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2014;10:372-380.
18. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2011;7:270-279.
19. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2011;7:280-292.
20. Storandt M, Head D, Fagan AM, Holtzman DM, Morris JC. Toward a multifactorial model of Alzheimer disease. *Neurobiology of aging* 2012;33:2262-2271.

21. Davson H. Review lecture. The blood-brain barrier. *The Journal of physiology* 1976;255:1-28.
22. Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 2008;57:178-201.
23. Winkler EA, Bell RD, Zlokovic BV. Central nervous system pericytes in health and disease. *Nat Neurosci* 2011;14:1398-1405.
24. Zenaro E, Piacentino G, Constantin G. The blood-brain barrier in Alzheimer's disease. *Neurobiology of Disease* 2017;107:41-56.
25. Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV. Establishment and Dysfunction of the Blood-Brain Barrier. *Cell* 2015;163:1064-1078.
26. Abbott NJ. Blood-brain barrier structure and function and the challenges for CNS drug delivery. *Journal of inherited metabolic disease* 2013;36:437-449.
27. Rosenberg GA. Neurological diseases in relation to the blood-brain barrier. *J Cereb Blood Flow Metab* 2012;32:1139-1151.
28. Tarasoff-Conway JM, Carare RO, Osorio RS, et al. Clearance systems in the brain-implications for Alzheimer disease. *Nature reviews Neurology* 2015;11:457-470.
29. Yamazaki Y, Kanekiyo T. Blood-Brain Barrier Dysfunction and the Pathogenesis of Alzheimer's Disease. *International journal of molecular sciences* 2017;18.
30. Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease--systematic review and meta-analysis. *Neurobiology of aging* 2009;30:337-352.
31. Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood-brain barrier: Implication for therapies in Alzheimer's disease. *CNS and Neurological Disorders - Drug Targets* 2009;8:16-30.
32. Bading JR, Yamada S, Mackic JB, et al. Brain clearance of Alzheimer's amyloid-beta40 in the squirrel monkey: a SPECT study in a primate model of cerebral amyloid angiopathy. *J Drug Target* 2002;10:359-368.
33. Deane R, Zlokovic BV. Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease. *Current Alzheimer research* 2007;4:191-197.
34. Wardlaw JM. Blood-brain barrier and cerebral small vessel disease. *J Neurol Sci* 2010;299:66-71.
35. Wardlaw JM, Makin SJ, Valdés Hernández MC, et al. Blood-brain barrier failure as a core mechanism in cerebral small vessel disease and dementia: evidence from a cohort study. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2017;13:634-643.
36. Cai Z, Wang C, He W, et al. Cerebral small vessel disease and Alzheimer's disease. *Clinical interventions in aging* 2015;10:1695-1704.
37. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *The Lancet Neurology* 2010;9:689-701.
38. Lammie GA. Hypertensive cerebral small vessel disease and stroke. *Brain pathology (Zurich, Switzerland)* 2002;12:358-370.
39. Vinters HV. Cerebral amyloid angiopathy. A critical review. *Stroke* 1987;18:311-324.
40. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesions. *The Lancet Neurology* 2012;11:272-282.
41. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *The Lancet Neurology* 2013;12:822-838.
42. Greenberg SM, Vernooij MW, Cordonnier C, et al. Cerebral microbleeds: a guide to detection and interpretation. *The Lancet Neurology* 2009;8:165-174.
43. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke* 2011;42:2672-2713.
44. Erickson MA, Banks WA. Blood-brain barrier dysfunction as a cause and consequence of Alzheimer's disease. *J Cereb Blood Flow Metab* 2013;33:1500-1513.
45. Viggars AP, Wharton SB, Simpson JE, et al. Alterations in the blood brain barrier in ageing cerebral cortex in relationship to Alzheimer-type pathology: a study in the MRC-CFAS population neuropathology cohort. *Neurosci Lett* 2011;505:25-30.
46. Hultman K, Strickland S, Norris EH. The APOE varepsilon4/varepsilon4 genotype potentiates vascular fibrin(ogen) deposition in amyloid-laden vessels in the brains of Alzheimer's disease patients. *J Cereb Blood Flow Metab* 2013;33:1251-1258.

47. Wisniewski HM, Kozlowski PB. Evidence for blood-brain barrier changes in senile dementia of the Alzheimer type (SDAT). *Ann N Y Acad Sci* 1982;396:119-129.
48. Rozemuller JM, Eikelenboom P, Kamphorst W, Stam FC. Lack of evidence for dysfunction of the blood-brain barrier in Alzheimer's disease: an immunohistochemical study. *Neurobiology of aging* 1988;9:383-391.
49. Alafuzoff I, Adolfsson R, Grundke-Iqbal I, Winblad B. Blood-brain barrier in Alzheimer dementia and in non-demented elderly. An immunocytochemical study. *Acta Neuropathol* 1987;73:160-166.
50. Hainsworth AH, Minett T, Andoh J, et al. Neuropathology of White Matter Lesions, Blood-Brain Barrier Dysfunction, and Dementia. *Stroke* 2017;48:2799-2804.
51. Bridges LR, Andoh J, Lawrence AJ, et al. Blood-brain barrier dysfunction and cerebral small vessel disease (arteriolosclerosis) in brains of older people. *J Neuropathol Exp Neurol* 2014;73:1026-1033.
52. Knudsen KA, Rosand J, Karluk D, Greenberg SM. Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. *Neurology* 2001;56:537-539.
53. Larsson HB, Courivaud F, Rostrup E, Hansen AE. Measurement of brain perfusion, blood volume, and blood-brain barrier permeability, using dynamic contrast-enhanced T(1)-weighted MRI at 3 tesla. *Magn Reson Med* 2009;62:1270-1281.
54. Montagne A, Barnes SR, Sweeney MD, et al. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* 2015;85:296-302.
55. van de Haar HJ, Burgmans S, Jansen JF, et al. Blood-Brain Barrier Leakage in Patients with Early Alzheimer Disease. *Radiology* 2016:152244.
56. Zhang CE, Wong SM, van de Haar HJ, et al. Blood-brain barrier leakage is more widespread in patients with cerebral small vessel disease. *Neurology* 2017;88:426-432.
57. Cho AH, Cho YP, Lee DH, et al. Reperfusion injury on magnetic resonance imaging after carotid revascularization. *Stroke* 2014;45:602-604.
58. Mamourian AC, Hoopes PJ, Lewis LD. Visualization of intravenously administered contrast material in the CSF on fluid-attenuated inversion-recovery MR images: an in vitro and animal-model investigation. *AJNR American journal of neuroradiology* 2000;21:105-111.
59. Merino JG, Latour LL, Tso A, et al. Blood-brain barrier disruption after cardiac surgery. *American Journal of Neuroradiology* 2013;34:518-523.
60. Kalaria RN, Akinyemi R, Ihara M. Does vascular pathology contribute to Alzheimer changes? *J Neurol Sci* 2012;322:141-147.
61. de la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. *The Lancet Neurology* 2004;3:184-190.

CHAPTER 2

WHITE MATTER HYPERINTENSITIES POTENTIATE HIPPOCAMPAL VOLUME REDUCTION IN NON-DEMENTED OLDER INDIVIDUALS WITH ABNORMAL AMYLOID- β .

Journal of Alzheimer's disease 2017;55(1):333-342.

Whitney M. Freeze, Heidi I. L. Jacobs, Ed H. B. M. Gronenschild, Jacobus F. A. Jansen, Saartje Burgmans, Pauline Aalten, Lies Clerx, Stephanie J. Vos, Mark A. van Buchem, Frederik Barkhof, Wiesje M. van der Flier, Marcel M. Verbeek, Marcel Olde Rikkert, Walter H. Backes and Frans R.

Verhey

ABSTRACT

Introduction: Cerebral small vessel disease (cSVD) and amyloid- β ($A\beta$) deposition often co-exist in (prodromal) dementia, and both types of pathology have been associated with neurodegeneration. We examined whether cSVD and $A\beta$ have independent or interactive effects on hippocampal volume (HV) in memory clinic patients.

Methods: We included 87 individuals with clinical diagnoses of Alzheimer's disease (AD) ($n=24$), mild cognitive impairment (MCI) ($n=26$), and subjective cognitive complaints (SCC) ($n=37$). cSVD magnetic resonance imaging markers included white matter hyperintensity (WMH) volume, lacunar infarct presence, and microbleed presence. $A\beta$ pathology was assessed as cerebrospinal fluid-derived $A\beta_{1-42}$ levels and dichotomized into normal or abnormal, and HV was determined by manual volumetric measurements. A linear hierarchical regression approach was applied for the detection of additive or interaction effects between cSVD and $A\beta$ on HV in the total participant group ($n=87$) and in the non-demented group (including SCC and MCI individuals only, $n=63$).

Results: The results revealed that abnormal $A\beta$ and lacunar infarct presence were both independently associated with lower HV in the non-demented individuals. Interestingly, $A\beta$ and WMH pathology interacted in the non-demented individuals, such that WMH had a negative effect on HV in individuals with abnormal CSF $A\beta_{42}$ levels, but not in individuals with normal CSF $A\beta_{42}$ levels. These associations were not present when individuals with AD were included in the analyses.

Conclusion: Our observations suggest that relatively early on in the disease process older individuals with abnormal $A\beta$ levels are at an increased risk of accelerated disease progression when concomitant cSVD is present.

BACKGROUND

Alzheimer disease (AD) and vascular dementia (VaD), the two most prevalent subtypes of dementia, are both characterized by progressive cognitive decline and neurodegeneration. Important pathophysiological processes underlying AD and VaD are cerebral amyloid-beta (A β) protein deposition and cerebral small vessel disease (cSVD) ^{1, 2}. Even though both types of dementia are often considered as separate entities, they share many risk factors and A β and cSVD pathology frequently co-occur ³⁻⁶. These observations suggest that the differentiation between AD and VaD can be difficult, and that the pure forms may rather represent two extremes of a pathologic continuum ⁷. Moreover, recent studies report positive associations between cerebral A β deposition and cSVD ⁸⁻¹², and the presence of mixed A β and cSVD pathology has been shown to increase the risk of developing dementia ¹³. These findings indicate that the underlying pathophysiological processes may be interdependent.

Hippocampal volume (HV) reduction is an important neurodegenerative marker for disease progression in dementia ¹⁴. Hippocampal atrophy has been independently associated with both A β pathology and cSVD in (prodromal) dementia ^{15, 16}. Interestingly, a recent animal study has pointed out that comorbid cerebrovascular damage and abnormal cerebral A β levels may interact in causing hippocampal cell degeneration ¹⁷. As such, presence of both elevated A β and cerebrovascular pathology may predispose individuals to being more vulnerable to neurodegeneration compared to individuals with only a single type of pathology. To date, it remains unclear whether A β deposition and cSVD interact in their detrimental effects on HV in individuals across the spectrum of dementia.

The aim of this study was to assess whether A β and cSVD pathologies interact in their effects on HV. We investigated cerebrospinal fluid A β_{42} levels as well as markers of cSVD pathology (white matter hyperintensities, lacunar infarcts and microbleeds) and HV in a representative sample of ageing memory clinic patients without large stroke. We hypothesized that A β and cSVD pathologies interacted on HV, such that the negative effect of either type of pathology on HV would be larger in the presence of the other type of pathology. We expected that these interactions would be present during the early stages of the disease, when dynamic and most pronounced changes in A β accumulation occur before reaching a plateau ². Post-hoc, we examined whether there was an interaction on cognition, a topic that is still debated in the current literature ¹⁸⁻²¹. Our results are important for the understanding of the cascade of pathological events in AD.

METHODS

Participants

Participants were recruited via three academic memory clinics (Maastricht University Medical Center, Radboud University Medical Center and VU University Medical Center) enrolled in the Dutch multi-center LeARN project. The process of recruitment and diagnostic classification has been described in detail elsewhere ²². In short, patients with subjective and/or objective memory complaints who were suspected of having a primary neurodegenerative disease were included. The present study selected all participants with available cross-sectional data on magnetic resonance imaging (MRI), lumbar puncture, and neuropsychological assessment. Individuals with macrovascular abnormalities including large cerebral infarction (diameter > 15mm) or hemorrhage (diameter > 10mm) were excluded (n=1). This resulted in a selection of 87 memory clinic patients, among which 37 individuals were diagnosed as having subjective cognitive complaints (SCC), 26 as having mild cognitive impairment (MCI) and 24 as having possible or probable AD. Diagnoses were made by experienced physicians based on conventional clinical criteria for MCI ²³ and AD ²⁴. Criteria for SCC diagnosis included presence of subjective cognitive complaints, but absence of objectivated cognitive impairment in any of the cognitive domains and absence of dementia. For all participants educational level and medical history were recorded. Hypertension was defined as current use of antihypertensive medication. Diabetes was defined as known history of diabetes type 1 or 2, and cardiovascular disease as known history of myocardial infarction, angina pectoris, carotid stenosis, dotter/stent placement and/or coronary bypass operation. The study was carried out in accordance with the rules and regulations of institutional research and ethics committees and written informed consent was obtained from all participants prior to participation ²².

MRI data acquisition and processing

At each medical center, MRI data were acquired on a 3.0 Tesla scanner. The scan protocol included a T1-weighted gradient-echo sequence, a fluid attenuated inversion recovery (FLAIR) sequence, as well as T2-weighted and susceptibility weighted imaging (SWI) sequences. The complete details of the acquisition and scan parameters per center can be found in the supplementary material (table S1). cSVD imaging markers were scored by expert raters who were trained to reach excellent intra-rater agreement compared to a gold standard (weighted kappa ≥ 0.85), unless otherwise specified. Raters were always blinded for clinical data when scoring neuroimaging cSVD markers and structural changes.

White matter hyperintensities

WMH volumes were quantified using a semi-automated method (in-house developed software package GIAN) ²⁵. For each medical center, an algorithm to classify WMH was trained separately, including the following steps. First, the FLAIR and T2 scans were corrected for intensity non-uniformities ²⁶, followed by a co-registration of the T2 scans with the corresponding FLAIR scans using FLIRT from the FMRIB's Software Library (FSL) version 5.0.4 (www.fmrib.ox.ac.uk/fsl). Next, the signal intensities of the FLAIR and T2 scans were normalized ²⁷. Then we selected five scans with a substantial amount of WMH. The axial FLAIR and T2-weighted images were displayed side by side allowing visual inspection and easy identification of WMH while they were traced manually. From these manual tracings, parameters were derived for automated classification of WMH. Finally, the actual segmentation was performed semi-automatically by clicking on each identified WMH region in each slice of the simultaneously displayed axial FLAIR and T2-weighted images, providing a seed point to initiate a region growing algorithm. Manual corrections were performed where necessary by a single rater (W.M.F.). Excellent inter- and intra-rater variability were computed for a subset (n=9) of scans that were also semi-automatically segmented by a second rater (S.B.) (intra class correlation both >0.98). Total WMH load was measured in cm³ and log-transformed to better approximate a normal distribution.

Lacunar infarcts

The number of lacunar infarcts was counted by characterizing lesions of minimal 3 to maximal 15mm in diameter with a signal intensity identical to CSF on FLAIR and T2-weighted images, possibly surrounded by a hyperintense rim on FLAIR images. Lacunar infarct count was dichotomized as 0 (absent) vs 1 (present).

Microbleeds

Cerebral microbleeds were defined as small, homogeneous, round foci of low signal intensity with associated blooming on SWI images of 2 to 10 mm in diameter ²⁸. Hypointensities at the site of the nucleus lentiformis, likely representing iron or calcium deposits, were excluded. Microbleed scores were dichotomized as 0 (absent) vs 1 (present).

Hippocampal volume

Coronal T1-weighted images were reformatted in a plane perpendicular to the long axis of the (left) hippocampus and HV was measured bilaterally by manual delineation as described previously ^{29, 30}. In short, the most anterior slice measured was the slice where the hippocampal formation was first

visible. The ventral border was formed by the white matter of the parahippocampal gyrus, while the dorsal border was formed by the amygdala on anterior slices and by CSF and choroid plexus on posterior slices. The HV included the dentate gyrus, cornu ammonis, subiculum, fimbria, and alveus. HV was computed by summing the delineated area of the region of interest on each slice and multiplying by the slice thickness. Delineation was done by expert raters with mean intra-rater variability below 5% and mean interrater variability below 8% (calculated from the ratio of the absolute difference in two measurements to the mean on a test set of scans ($n=10$)). HV was measured in cm^3 and expressed as the sum volume of the left and right hippocampus.

Intracranial volume

Intracranial volume (ICV) was derived from the T1 scans by using the SIENAX tool from FSL. ICV was measured in cm^3 .

CSF analysis

CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space, and stored at -20°C in polypropylene tubes at each center. Samples of Maastricht and Nijmegen were transported to Amsterdam on dry ice for analysis. CSF $\text{A}\beta_{42}$ levels were quantified with commercially available INNOTEST sandwich enzyme-linked immunosorbent assays (Fujirebio formerly Innogenetics, Ghent, Belgium) as previously described³¹. To classify individuals according to CSF $\text{A}\beta_{42}$ status (normal vs abnormal), routinely used validated cut-offs were applied, which could differentiate between subjects with SCC and AD-type dementia with 85% sensitivity (abnormal CSF $\text{A}\beta_{42} \leq 550 \text{ pg/ml}$)³².

Cognition

The Mini Mental State Examination (MMSE)³³ was used to assess cognitive impairment, and the Clinical Dementia Rating scale (CDR) was used to measure cognitive status. Neuropsychological testing for specific cognitive domains included quantitative tests to measure delayed memory recall (verbal 15 word learning test delayed word recall³⁴ [number of correctly reproduced words 20 minutes after the last learning trial]), language (Verbal Fluency Test³⁵ [number of correctly named animals within 1 minute]), and executive functioning (Stroop Color-Word Test³⁶ [interference score taken as outcome measure³⁷]). Raw test scores were converted to z-scores, adjusted for age, sex and education based on normative data^{34, 35, 37}.

Statistical analysis

All analyses were conducted using SPSS statistical software (version 22.0; IBM Corp., Armonk, NY). A threshold of $\alpha < 0.05$ was used to determine statistical significance. Participant characteristics were described for each diagnostic group (SCC, MCI, AD). One-way analyses of variance (ANOVAs) were performed to assess group differences for continuous variables, and chi-squared tests or Fisher's exact tests (where applicable) to test for differences in proportions between groups for categorical variables.

Interaction and additive effects of A β status and cSVD markers on HV

We conducted linear hierarchical regression analyses to investigate our aims. First a baseline step 1 model was built, with covariates age, sex, ICV, center, and MMSE score (to correct for the degree of cognitive impairment) included. In step 2, A β_{42} status was added to the baseline model together with WMH, lacunar infarcts, or microbleeds, and their interaction was added in the third step. Step 2 and 3 were performed for each cSVD marker separately. We applied these analyses to the total participant group, and to the non-demented group (including only SCC and MCI participants). We applied false discovery rate control to the p-values to correct for multiple comparisons of different predictor variables (three different cSVD markers as predictor of HV).

Post-hoc interaction and additive effects of A β and cSVD markers on cognition

We performed the same analyses with z-transformed cognitive measures of delayed memory recall, language, and executive functioning as dependent variables. Because z-scores were already corrected for age, sex, and education, only MMSE score and center were included as covariates in step 1. Because MMSE score is associated with the cognitive outcome measures and may as such explain a substantial amount of the variance, we also did the analyses with only center as a covariate in step 1 to see whether this changed the results. We applied false discovery rate control to the p-values to correct for multiple comparisons of different outcome variables (three different cognitive tests)³⁸.

RESULTS

Participant characteristics stratified by clinical status are summarized in Table 1. Individuals with AD were less often recruited via the MUMC and were more likely to have abnormal A β_{42} levels compared with individuals with SCC. Individuals with AD or MCI were older, had smaller HVs, and had larger WMH volumes compared with participants with SCC. Individuals with AD had lower A β_{42} levels and less often lacunar infarction compared with all other individuals. CDR scores

were higher in individuals with AD as compared with all other participants, whereas MMSE, delayed memory recall, language, and executive functioning scores were lower. Furthermore, CDR scores were higher, and MMSE and delayed memory recall scores were lower in participants with MCI as compared with participants with SCC.

Table 1: Participant characteristics

	SCC	MCI	AD
Demographics	(n=37)	(n=26)	(n=24)
Age, mean(SD), y	63.70(9.3)	69.15(8.4) ^a	69.96(8.7) ^a
Female sex, No(%)	11(30)	7(27)	9(38)
Education, mean(SD), y	11.97(3.8)	11.69(3.1)	10.71(3.2)
MUMC, No(%)	20(54)	11(42)	5(21) ^a
NUMC, No(%)	10(27)	9(35)	9(38)
VUMC, No(%)	7(19)	6(23)	10(42)
Vascular risk factors No(%)			
Hypertension	13(35)	9(35)	7(29)
Diabetes mellitus	1(3)	4(15)	4(17)
Cardiovascular disease	2(5)	2(8)	5(21)
MRI characteristics			
WMH, mean(SD), cm ³	5.80(5.5)	10.67(10.1) ^a	10.08(9.2) ^a
Lacunes present, No(%)	8(22)	7(27)	0(0) ^{ab}
Microbleeds present, No(%)	10(27)	7(27)	6(25)
HV, mean (SD), cm ³	6.7(0.8)	5.8(1.0) ^a	6.1(1.0) ^a
ICV, mean(SD), cm ³	1951(171)	1933(209)	1901(135)
CSF biomarker level			
A β ₄₂ , mean(SD), pg/mL	776(249)	737(284)	540(173) ^{ab}
Abnormal A β ₄₂ , No(%)	8(22)	10(38)	13(54) ^a
Cognition			
MEM z-score, mean(SD)	-0.48(1.1)	-1.96(1.0) ^a	-2.57(1.1) ^{ab}
LA z-score, mean(SD) [§]	-0.59(0.7)	-0.92(0.9)	-1.50(0.7) ^{ab}
EF z-score, mean(SD) [§]	-0.50(1.7)	-0.10(1.4)	-2.52(3.7) ^{ab}
MMSE, mean(SD)	28.2(1.4)	27.2(2.2) ^a	23.6(2.7) ^{ab}
CDR, mean(SD) [§]	0.35(0.3)	0.50(0.0) ^a	0.84(0.2) ^{ab}

Abbreviations: SCC, subjective cognitive complaints; MCI, mild cognitive impairment; AD, Alzheimer disease; MUMC, Maastricht University Medical Center; NUMC, Nijmegen University Medical Center; WMH, white matter hyperintensities; HV, hippocampal volume; A β , amyloid-beta; MEM, delayed memory recall; LA, language; EF, executive functioning; MMSE, mini-mental state examination; CDR, clinical dementia rating scale; SD, standard deviation

^ap<0.05 vs SCC; ^bp<0.05 vs MCI; [§]Data were missing, LA z-score n=86; EF z-score n=80; CDR n=81

Interaction and additive effects of A β and cSVD markers on HV

Table 2 shows the additive and interaction effects of CSF A β ₄₂ status and different cSVD markers on HV. There were no independent or additive effects in the whole group analyses. In the non-demented group, abnormal CSF A β ₄₂ and lacunar infarct presence were both independently and additively associated with lower HV volume. Moreover, an interaction effect was present between CSF A β ₄₂ and WMH volume in the non-demented group, such that larger WMH volumes were

associated with lower HV volumes in individuals with abnormal CSF A β_{42} , but not in individuals with normal CSF A β_{42} (figure 1). No interactions were found in the whole group analyses.

Figure 1: The association between HV and WMH volume varies according to A β_{42} status (dichotomized). Higher WMH volumes are associated with lower HV in individuals with abnormally low CSFA β_{42} levels ($n=18$; $r=-0.53$, $p<0.05$), but not in individuals with normal CSFA β_{42} levels ($n=45$; $r=-0.12$, n.s.). Abbreviations: A β , amyloid-beta; HV, hippocampal volume; WMH, white matter hyperintensities; n.s., not significant. Unadjusted Pearson's correlations are reported.

Table 2: Additive and interactive effects of cSVD markers and CSF A β_{42} on HV

	Whole group			Non-demented individuals only		
	<u>Additive impact</u>		<u>Interaction</u>	<u>Additive impact</u>		<u>Interaction</u>
	cSVD	Aβ_{42}	cSVDx$A\beta_{42}$	cSVD	Aβ_{42}	cSVDx$A\beta_{42}$
WMH	-0.01	-0.11	-1.98	-0.19	-0.31**	-3.63*
Lacunes	-0.20	-0.16	0.13	-0.30*	-0.37**	0.25
MB	0.11	-0.12	0.08	0.16	-0.30*	0.20

Values represent standardized beta coefficients.

Abbreviations: A β , amyloid-beta; WMH, white matter hyperintensities (log transformed); MB, microbleeds; HV, hippocampal volume; cSVD, cerebral small vessel disease.

* $p<0.05$; ** $p<0.01$

Interaction and additive effects of $A\beta$ and cSVD markers on cognition

In the whole group and in the non-demented group, WMH volume was negatively associated with delayed memory recall performance (supplementary material table S2). No other independent effects of cSVD markers or $A\beta_{42}$ were observed. There were no interaction effects between CSF $A\beta_{42}$ and the cSVD imaging markers on any cognitive measure in the whole group or in the non-demented group (supplementary material table S2a-c). No changes occurred in the results when MMSE score was excluded from the model as a covariate.

DISCUSSION

The main objective of this study was to assess whether $A\beta$ and cSVD interact on HV in older individuals ranging from cognitively normal to demented. Our results indicated that abnormal CSF $A\beta_{42}$ levels and cSVD burden in the form of lacunar infarct presence were both independently associated with smaller HV in non-demented older individuals. Although independent effects of WMH volume or MB presence on HV were absent in all analyses, the results did reveal an interaction effect between $A\beta_{42}$ pathology and WMH volume in the non-demented group. Interestingly, WMH had a negative relationship with HV in individuals with abnormal $A\beta_{42}$ levels, but such associations were absent in individuals with normal $A\beta_{42}$ levels. This interaction effect disappeared when individuals with dementia were included in the analyses. Importantly, these findings extend the current literature by showing that especially in the early stages of the disease process, individuals with $A\beta$ pathology may be at increased risk for accelerated disease progression in the presence of comorbid cSVD pathology.

Our results are in line with recent work showing that the effects of cerebrovascular risk factors on cortical atrophy are influenced by the amount of $A\beta$ pathology³⁹. The authors found that the negative effects of vascular risk on cortical thickness, especially in AD vulnerable regions, were increased when higher amounts of cerebral $A\beta$ deposition were present. This interaction effect was found in a similar population sample of non-demented individuals with normal and mildly impaired cognition. Our findings add new insights by showing that $A\beta$ pathology facilitates the impact of actual small vessel damage on neurodegeneration within the hippocampus, a crucial structure that is also highly vulnerable to AD.

However, the existing literature remains inconclusive. Hohman et al. investigated the interplay between $A\beta$ levels and cerebrovascular risk, and indicated a reversed interaction effect, such that cerebrovascular risk was strongest related to hippocampal atrophy in individuals without $A\beta$ pathology⁴⁰. This reversed interaction effect might have been influenced by inclusion of clinical-stage dementia. In our analyses, inclusion of individuals with dementia made the interaction

effect between cSVD and A β disappear. It could be that at a later disease stage, A β pathology related processes take over and the role of vascular factors becomes smaller ⁴¹. Another recent study found no interaction between A β levels and cerebrovascular pathology in cognitively normal individuals with hippocampal atrophy as outcome measure ⁴². Although this was a well-designed longitudinal study, it could be that such interactions are not detectable at the earliest stages preceding subjective or objective cognitive problems. Additional work in population-based samples is needed to clarify the dynamic interplay between vascular damage and A β from normal aging to dementia.

It remains unknown whether vascular and A β pathology directly influence each other in their rates of pathologic progression, or whether these are simply distinct processes occurring in parallel. In case of the first scenario, cSVD pathology may lead to reduced parenchymal A β clearance or increased deposition at the level of the neurovascular unit, while A β accumulation in and around the cerebral vessels may in turn lead to small vessel damage. Such interactions have been reported in hypertensive rats ¹². These possible mechanisms need further exploration since they might have important implications for the diagnostic classification of AD and VaD, suggesting that a continuous approach might be more appropriate than separate classification into AD and VaD. In case of the independent processes scenario, it can be hypothesized that A β related processes reduce compensatory brain reserve ^{43, 44} and lower the threshold for (further) neurodegeneration due to cerebrovascular damage. Importantly, the interaction effect between A β and WMH found in the present study demonstrates that WMH pathology impacts hippocampal neurodegeneration only when abnormal A β levels are already present. This suggests that individuals with both types of pathology are at increased risk of accelerated disease progression, which is in line with earlier findings that individuals with cerebrovascular damage are at increased risk of developing AD ^{13, 45, 46}.

The fact that A β pathology was independently associated with smaller HV in the non-demented sample is in accordance with previous studies consistently showing negative associations between cerebral A β deposition and HV at early disease stages ^{47, 48}. Besides A β pathology, lacunar infarct presence was independently and negatively associated with HV. This finding suggests that hippocampal neurodegeneration can also be independently driven by vascular factors ¹⁶. This effect was absent in the whole group analyses because none of the AD individuals displayed signs of lacunar infarction. We did not find any associations between HV and cerebral MBs or WMH volume. Although MBs have previously been associated with HV reduction ⁴⁹, our sample included only a limited number (around 25%) of participants that displayed these lesions. As such, our analyses may lack statistical power and sensitivity to detect associations between MBs and HV.

Post-hoc, we examined a possible interplay between A β and cSVD on cognition. In line with previous studies, we found no interaction effects between A β and cSVD on various cognitive domains.^{19, 50, 51} Although emerging evidence suggests that dementia is more likely to occur when vascular and A β pathology coexist^{13, 45}, it may be the case that the effects on cognition are mediated by neurodegenerative factors. For example, previous work indicated that the association between A β and cognitive decline is mediated by gray matter atrophy and glucose metabolism⁵². Others found that brain atrophy interacts with cSVD in affecting cognitive decline⁵³. Thus, when not taking other factors into account, A β and cSVD pathology may have independent or additive effects on cognition. Recent work supports the additive effect hypothesis by showing that cognitively normal individuals with both cerebrovascular and amyloid pathology displayed faster cognitive decline that seemed additive compared to the rate in individuals with only one type of pathology²¹. However, a recent study in individuals with subcortical vascular dementia reported interactions between cSVD and A β pathology on longitudinal cognitive outcomes²⁰. As such, the subtle interactive effects of cSVD and A β may only become apparent at advanced disease stage and may only be detectable when cognition is measured longitudinally. Further studies are needed to clarify this matter.

Our results showed a relationship between WMH volume and delayed memory recall in the whole sample and in the non-demented individuals, which confirms the well-established association between cerebrovascular injury and cognition²¹. However, no associations between cognition and the other cSVD imaging markers were found, which may be explained by the relatively low prevalence of these markers (~25%). While the association of A β pathology with cognition is less pronounced^{54, 55}, previous studies do indicate subtle independent associations with cognitive performance^{18, 19}. It is therefore important not to overlook the possible effects of both A β and cerebrovascular disease when studying cognition in dementia. However, the effects of neurodegeneration, glucose metabolism, and other possible influencing factors should be taken into account.

Strengths of this study are the multi-center nature and representativeness of our study sample for general memory-clinic visiting individuals. Furthermore, the exclusion of large strokes enabled us to study the effects of mild cSVD in interaction with A β , although this may be a limitation to the generalizability of our results to populations with large vessel disease. The range in cognitive impairment allowed us to investigate our aims across the whole spectrum of dementia, from cognitively normal to mild dementia. Pitfalls of this study include its restricted sample size, the relatively low diversity of cSVD severity (The absence of lacunar infarcts in the AD group may form a potential sample bias in this study), and the cross-sectional nature of the data, which makes

it impossible to make direct causal inferences. The multi-center nature may also be a confounding factor, because the scans are acquired on three different MR systems with different scan parameters. As such, the WMH volume calculation might have been influenced by center. We tried to correct for this by calculating separate algorithms for each center and by adding center as a covariate in the statistical models.

Taken together, the findings in this study suggest that A β pathology and cerebrovascular damage can both independently and synergistically facilitate hippocampal neurodegeneration in non-demented older individuals. Our results showed that cSVD in the form of lacunar infarcts was associated with smaller HVs in individuals with subjective to mild cognitive problems. Likewise, these individuals were more likely to have lower HV when abnormal cerebral A β deposition was present. These associations were independent, meaning that their negative effects of A β pathology and lacunar infarcts on HV were additive. In contrast, an interaction effect on HV was present between abnormal A β levels and WMH in the non-demented individuals. That is, WMH volume had no effect on HV in individuals with normal A β levels, while HV reduction was (even further) amplified in individuals with abnormal cerebral A β deposition when comorbid cerebrovascular disease in the form of WMH was present. We found no associations or interactions when individuals with AD were included in the analyses. Importantly, our results suggest that associations and interactions between A β and cSVD pathologies in relation to HV take place at the earlier stages of the disease process when cognitive impairment is only mild or not yet detectable. At this early stage, brain reserve may play a protective role, while during clinical disease stage other A β -related processes such as advanced neurodegeneration and/or tau pathology may have impacted this reserve. The observations in this study underline the importance of taking both cerebrovascular disease and A β deposition into account when studying cerebral atrophy in dementia. Vascular care in early memory clinic patients may form an important opportunity to slow down disease progression.

REFERENCES

1. Esiri MM, Wilcock GK, Morris JH. Neuropathological assessment of the lesions of significance in vascular dementia. *Journal of neurology, neurosurgery, and psychiatry* 1997;63:749-753.
2. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *The Lancet Neurology* 2013;12:207-216.
3. Attems J, Jellinger KA. The overlap between vascular disease and Alzheimer's disease--lessons from pathology. *BMC medicine* 2014;12:206.
4. Carotenuto A, Rea R, Colucci L, et al. Late and early onset dementia: what is the role of vascular factors? A retrospective study. *J Neurol Sci* 2012;322:170-175.
5. Jellinger KA. The enigma of mixed dementia. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2007;3:40-53.
6. Kalara RN, Akinyemi R, Ihara M. Does vascular pathology contribute to Alzheimer changes? *J Neurol Sci* 2012;322:141-147.
7. de la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. *The Lancet Neurology* 2004;3:184-190.
8. Grimmer T, Faust M, Auer F, et al. White matter hyperintensities predict amyloid increase in Alzheimer's disease. *Neurobiology of aging* 2012;33:2766-2773.
9. Kaffashian S, Tzourio C, Soumare A, et al. Plasma beta-amyloid and MRI markers of cerebral small vessel disease: Three-City Dijon study. *Neurology* 2014;83:2038-2045.
10. Kester MI, Goos JD, Teunissen CE, et al. Associations between cerebral small-vessel disease and Alzheimer disease pathology as measured by cerebrospinal fluid biomarkers. *JAMA neurology* 2014;71:855-862.
11. Park JH, Seo SW, Kim C, et al. Pathogenesis of cerebral microbleeds: In vivo imaging of amyloid and subcortical ischemic small vessel disease in 226 individuals with cognitive impairment. *Annals of neurology* 2013;73:584-593.
12. Schreiber S, Drukarch B, Garz C, et al. Interplay between age, cerebral small vessel disease, parenchymal amyloid-beta, and tau pathology: longitudinal studies in hypertensive stroke-prone rats. *Journal of Alzheimer's disease : JAD* 2014;42 Suppl 3:S205-215.
13. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* 2007;69:2197-2204.
14. Jack CR, Jr., Petersen RC, Xu YC, et al. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. *Neurology* 1999;52:1397-1403.
15. Guzman VA, Carmichael OT, Schwarz C, Tosto G, Zimmerman ME, Brickman AM. White matter hyperintensities and amyloid are independently associated with entorhinal cortex volume among individuals with mild cognitive impairment. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2013;9:S124-131.
16. Kim GH, Lee JH, Seo SW, et al. Hippocampal volume and shape in pure subcortical vascular dementia. *Neurobiology of aging* 2015;36:485-491.
17. Amtul Z, Nikolova S, Gao L, et al. Comorbid Abeta toxicity and stroke: hippocampal atrophy, pathology, and cognitive deficit. *Neurobiology of aging* 2014;35:1605-1614.
18. Lee MJ, Seo SW, Na DL, et al. Synergistic effects of ischemia and beta-amyloid burden on cognitive decline in patients with subcortical vascular mild cognitive impairment. *JAMA psychiatry* 2014;71:412-422.
19. Park JH, Seo SW, Kim C, et al. Effects of cerebrovascular disease and amyloid beta burden on cognition in subjects with subcortical vascular cognitive impairment. *Neurobiology of aging* 2014;35:254-260.
20. Ye BS, Seo SW, Kim JH, et al. Effects of amyloid and vascular markers on cognitive decline in subcortical vascular dementia. *Neurology* 2015;85:1687-1693.
21. Vemuri P, Lesnick TG, Przybelski SA, et al. Vascular and amyloid pathologies are independent predictors of cognitive decline in normal elderly. *Brain : a journal of neurology* 2015;138:761-771.
22. Handels RL, Aalten P, Wolfs CA, et al. Diagnostic and economic evaluation of new biomarkers for Alzheimer's disease: the research protocol of a prospective cohort study. *BMC neurology* 2012;12:72.
23. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association

- workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2011;7:270-279.
24. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34:939-944.
25. Jacobs HI, Clerx L, Gronenschild EH, Aalten P, Verhey FR. White matter hyperintensities are positively associated with cortical thickness in Alzheimer's disease. *Journal of Alzheimer's disease : JAD* 2014;39:409-422.
26. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE transactions on medical imaging* 1998;17:87-97.
27. Nyul LG, Udupa JK. On standardizing the MR image intensity scale. *Magn Reson Med* 1999;42:1072-1081.
28. Greenberg SM, Vernooij MW, Cordonnier C, et al. Cerebral microbleeds: a guide to detection and interpretation. *The Lancet Neurology* 2009;8:165-174.
29. van de Pol LA, van der Flier WM, Korf ES, Fox NC, Barkhof F, Scheltens P. Baseline predictors of rates of hippocampal atrophy in mild cognitive impairment. *Neurology* 2007;69:1491-1497.
30. Jack CR, Jr. MRI-based hippocampal volume measurements in epilepsy. *Epilepsia* 1994;35 Suppl 6:S21-29.
31. Vos SJ, Visser PJ, Verhey F, et al. Variability of CSF Alzheimer's disease biomarkers: implications for clinical practice. *PloS one* 2014;9:e100784.
32. Mulder C, Verwey NA, van der Flier WM, et al. Amyloid-beta(1-42), total tau, and phosphorylated tau as cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease. *Clin Chem* 2010;56:248-253.
33. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research* 1975;12:189-198.
34. Van der Elst W, van Boxtel MP, van Breukelen GJ, Jolles J. Rey's verbal learning test: normative data for 1855 healthy participants aged 24-81 years and the influence of age, sex, education, and mode of presentation. *Journal of the International Neuropsychological Society : JINS* 2005;11:290-302.
35. Van der Elst W, Van Boxtel MP, Van Breukelen GJ, Jolles J. Normative data for the Animal, Profession and Letter M Naming verbal fluency tests for Dutch speaking participants and the effects of age, education, and sex. *Journal of the International Neuropsychological Society : JINS* 2006;12:80-89.
36. Stroop JR. Studies of interference in serial verbal reactions. *J Exp Psychol* 1935;18(6):643-662.
37. Van der Elst W, Van Boxtel MP, Van Breukelen GJ, Jolles J. The Stroop color-word test: influence of age, sex, and education; and normative data for a large sample across the adult age range. *Assessment* 2006;13:62-79.
38. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)* 1995:289-300.
39. Villeneuve S, Reed BR, Madison CM, et al. Vascular risk and A β interact to reduce cortical thickness in AD vulnerable brain regions. *Neurology* 2014;83:40-47.
40. Hohman TJ, Samuels LR, Liu D, et al. Stroke risk interacts with Alzheimer's disease biomarkers on brain aging outcomes. *Neurobiology of aging* 2015;36:2501-2508.
41. Chui HC, Zarow C, Mack WJ, et al. Cognitive impact of subcortical vascular and Alzheimer's disease pathology. *Annals of neurology* 2006;60:677-687.
42. Vemuri P, Knopman DS. The role of cerebrovascular disease when there is concomitant Alzheimer disease. *Biochimica et biophysica acta* 2016;1862:952-956.
43. Mormino EC. The relevance of beta-amyloid on markers of Alzheimer's disease in clinically normal individuals and factors that influence these associations. *Neuropsychology review* 2014;24:300-312.
44. Huijbers W, Mormino EC, Schultz AP, et al. Amyloid-beta deposition in mild cognitive impairment is associated with increased hippocampal activity, atrophy and clinical progression. *Brain : a journal of neurology* 2015;138:1023-1035.
45. van der Flier WM, Middelkoop HA, Weverling-Rijnsburger AW, et al. Interaction of medial temporal lobe atrophy and white matter hyperintensities in AD. *Neurology* 2004;62:1862-1864.

46. Tosto G, Zimmerman ME, Hamilton JL, Carmichael OT, Brickman AM. The effect of white matter hyperintensities on neurodegeneration in mild cognitive impairment. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2015;11:1510-1519.
47. Chetelat G, Villemagne VL, Bourgeat P, et al. Relationship between atrophy and beta-amyloid deposition in Alzheimer disease. *Annals of neurology* 2010;67:317-324.
48. Mormino EC, Kluth JT, Madison CM, et al. Episodic memory loss is related to hippocampal-mediated beta-amyloid deposition in elderly subjects. *Brain : a journal of neurology* 2009;132:1310-1323.
49. Chowdhury MH, Nagai A, Bokura H, Nakamura E, Kobayashi S, Yamaguchi S. Age-related changes in white matter lesions, hippocampal atrophy, and cerebral microbleeds in healthy subjects without major cerebrovascular risk factors. *Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association* 2011;20:302-309.
50. Marchant NL, Reed BR, DeCarli CS, et al. Cerebrovascular disease, beta-amyloid, and cognition in aging. *Neurobiology of aging* 2012;33:1006.e1025-1036.
51. Marchant NL, Reed BR, Sanossian N, et al. The aging brain and cognition: contribution of vascular injury and abeta to mild cognitive dysfunction. *JAMA neurology* 2013;70:488-495.
52. Ewers M, Insel P, Jagust WJ, et al. CSF biomarker and PIB-PET-derived beta-amyloid signature predicts metabolic, gray matter, and cognitive changes in nondemented subjects. *Cerebral cortex (New York, NY : 1991)* 2012;22:1993-2004.
53. Jokinen H, Lipsanen J, Schmidt R, et al. Brain atrophy accelerates cognitive decline in cerebral small vessel disease: the LADIS study. *Neurology* 2012;78:1785-1792.
54. Aizenstein HJ, Nebes RD, Saxton JA, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. *Arch Neurol* 2008;65:1509-1517.
55. Villemagne VL, Pike KE, Chetelat G, et al. Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease. *Annals of neurology* 2011;69:181-192.

SUPPLEMENTARY MATERIAL

Table S1: MRI acquisition parameters according to Medical Center

	Plane	TR (ms)	TE (ms)	T1 (ms)	Flip angle (°)	Matrix size (mm)	Voxel size (mm)	No of slices
MUMC Philips Achieva 3.0T								
T1	Sagittal	8.2	3.7		8°	240x240	1.00x1.00x1.00	180
T2	Axial	2500	100		90°	512x512	0.50x0.50x3.00	48
FLAIR	Axial	11000	125	2800	90°	512x512	0.50x0.50x3.00	48
SWI	Axial	5300	25		90°	512x512	0.50x0.50x3.00	48
NUMC Siemens TrioTim 3.0T								
T1	Sagittal	2300	4.7	1100	90°	256x256	1.00x1.00x1.00	192
T2	Axial	5830	120		90°	384x324	0.65x0.65x3.00	48
FLAIR	Axial	12000	121	2879	90°	384x324	0.65x0.65x3.00	48
SWI	Axial	29	20		90°	320x270	0.78x0.78x3.00	48
VUMC GE Signa HDxt 3.0T								
T1	Sagittal	7.8	3.0	450	90°	256x256	0.94x0.94x1.00	176
T2	Axial	8520	113		90°	512x512	0.49x0.49x3.00	48
FLAIR	Sagittal	8000	125	2348	90°	256x256	0.98x0.98x1.20	132
SWI	Axial	5300	25		90°	512x512	0.49x0.49x3.00	48

Note: None of the centers included a slice gap in any sequence

Abbreviations: mm, milli meters; ms, milli seconds; TR, repetition time; TI, inversion time; MUMC, Maastricht university medical center; NUMC, Nijmegen university medical center; VUMC, VU medical center; FLAIR, fluid-attenuated inversion recovery; SWI, susceptibility weighted imaging.

Table S2a: Additive and interactive effects of cSVD markers and CSF A β_{42} on Delayed memory recall

	Whole group (n=87)			Non-demented individuals only (n=63)		
	<u>Additive impact</u>		<u>Interaction</u>	<u>Additive impact</u>		<u>Interaction</u>
	cSVD marker	A β_{42}	cSVDx $A\beta_{42}$	cSVD	A β_{42}	cSVDx $A\beta_{42}$
WMH	-0.33***	-0.11	-0.19	-0.41***	-0.21	-0.79
Lacunes	-0.11	-0.15	0.05	-0.21	-0.26	0.12
MB	-0.04	-0.13	-0.07	-0.01	-0.22	0.11

Table S2b: Additive and interactive effects of cSVD markers and CSF A β_{42} on Language

	Whole group (n=86)			Non-demented individuals only (n=63)		
	<u>Additive impact</u>		<u>Interaction</u>	<u>Additive impact</u>		<u>Interaction</u>
	cSVD marker	A β_{42}	cSVDx $A\beta_{42}$	cSVD	A β_{42}	cSVDx $A\beta_{42}$
WMH	-0.04	0.05	-1.06	-0.06	0.03	-1.86
Lacunes	-0.06	0.03	0.08	-0.18	-0.01	0.15
MB	-0.03	0.05	-0.29	0.03	0.03	-0.17

Table S2c: Additive and interactive effects of cSVD markers and CSF A β_{42} on Executive functioning

	Whole group (n=80)			Non-demented individuals only (n=58)		
	<u>Additive impact</u>		<u>Interaction</u>	<u>Additive impact</u>		<u>Interaction</u>
	cSVD marker	A β_{42}	cSVDx $A\beta_{42}$	cSVD	A β_{42}	cSVDx $A\beta_{42}$
WMH	0.17	-0.13	2.49	0.26	-0.15	2.25
Lacunes	0.01	-0.11	-0.14	-0.09	-0.16	-0.37
MB	0.09	-0.10	-0.24	0.23	-0.14	0.19

Note: Values represent standardized beta coefficients.

Abbreviations: A β , amyloid-beta; CSF, cerebrospinal fluid; WMH, white matter hyperintensities (log transformed); MB, microbleeds; cSVD, cerebral small vessel disease.

***p<0.001.

CHAPTER 3

ON THE LINK BETWEEN BLOOD-BRAIN BARRIER LEAKAGE, WHITE MATTER HYPERINTENSITIES, NEURODEGENERATION, AND COGNITION.

Submitted.

Whitney M. Freeze, Heidi I.L. Jacobs, Joost J. de Jong, Inge C.M. Verheggen, Ed H. B. M.
Gronenschild, Walter M. Palm, Erik I. Hoff, Joanna M. Wardlaw, Jacobus F.A. Jansen, Frans R.
Verhey, and Walter H. Backes

ABSTRACT

Introduction: Blood-brain barrier (BBB) disruption is considered an important underlying process in both cerebral small vessel disease and Alzheimer disease pathology, though its precise role and clinical relevance need to be elucidated.

Methods: In 80 participants with normal cognition, mild cognitive impairment, and Alzheimer disease, regional BBB leakage was assessed with dynamic contrast-enhanced MRI. Associations between BBB leakage and hippocampal volume, white matter hyperintensity volume, and cognition were examined using multiple regression and mediation analyses across all individuals.

Results: Leakage within the gray as well as white matter was positively associated with white matter hyperintensity volume, but not with hippocampal volume. A negative association between white matter BBB disruption and information processing speed performance was mediated by white matter hyperintensity volume.

Conclusion: BBB leakage is positively related to white matter hyperintensity volume, which may form an underlying mechanism linking BBB disruption to slower information processing speed in older individuals with normal cognition, mild cognitive impairment and Alzheimer disease.

BACKGROUND

Alzheimer disease (AD) is characterized by amyloid- β and neurodegenerative pathology ¹. However, several neuroimaging and pathological studies have suggested that vascular pathology in the form of cerebral small vessel disease (cSVD) also plays a significant role in the AD disease cascade that ultimately leads to cognitive decline and functional impairment ^{2,5}. A pathological process that could link microvascular damage and AD is blood-brain barrier (BBB) disruption ^{6,7}. The BBB controls the homeostasis of the neuronal environment, which is essential for neuronal vitality and signaling ⁸. Disruption of the BBB may lead to an ill-conditioned brain tissue environment with eventual neuronal dysfunction, tissue damage or even cell loss.

Quantitative information about localized functional BBB disruption within the living human brain can be obtained with dynamic contrast-enhanced MRI (DCE-MRI). A number of recent studies have applied this technique to demonstrate involvement of BBB disruption in normal aging ⁹, cSVD ^{10,11}, mild cognitive impairment (MCI) ⁹, and AD ¹². These studies suggest involvement of BBB disruption in the pathophysiology of cSVD-related MRI abnormalities such as white matter hyperintensities (WMH) ^{13,14}, but also contributions of BBB disruption and microangiopathy to early neurodegenerative processes associated with AD ¹⁵. The associations between BBB disruption, cSVD, neurodegeneration, aging, cognition and disease stage are extremely complex and not yet well understood ⁶. Further clarification of these associations is pivotal to increase our understanding of vascular contributions to AD and to find novel diagnostic and therapeutic strategies.

The primary aim of this study was to examine links between quantitative measures of BBB disruption (i.e. leakage rate and volume), cSVD burden (i.e. WMH volume), neurodegeneration (i.e. hippocampal volume (HV)), and cognition in individuals within the range from normal cognition to AD-type dementia. Because BBB disruption directly reflects vascular integrity, we (i) hypothesized that increased cSVD burden (i.e. larger WMH volume) was associated with increased BBB disruption in both gray and white matter. Because previous work has suggested that BBB disruption is typically increased within the hippocampus in individuals with MCI ⁹, we (ii) hypothesized that advanced neurodegeneration (i.e. smaller HV) was associated with increased BBB disruption within the hippocampus. Finally, to examine the potential clinical relevance of regional BBB disruption, we (iii) performed mediation analyses to examine whether HV mediated the potential associations between memory performance and hippocampal BBB disruption, and whether WMH volume mediated the association between information processing speed performance and white matter BBB disruption. To obtain representative variation in WMH volume, HV, and cognitive function across the AD spectrum, cognitively normal older individuals

as well as memory clinic patients with various degrees of cognitive impairment were included in this study.

METHODS

Participant selection

Memory clinic patients were recruited at Maastricht University Medical Center and Zuyderland Medical Center Heerlen. All participants were included between November 2014 and October 2017. Inclusion criteria for patients were a clinical diagnosis of either subjective cognitive decline (SCD)¹⁶, mild cognitive impairment (MCI)¹⁷ or AD¹⁸, and mini-mental state examination (MMSE) score ≥ 20 . Cognitively normal controls were enrolled through an advertisement in the local newspaper and an online advertisement. Control participants had no cognitive complaints, were not known as a patient from a memory clinic, and had an MMSE score ≥ 27 . Inclusion criteria for all participants were age ≥ 55 years and eligibility for undergoing 3 Tesla MRI and gadolinium contrast medium administration. Exclusion criteria and participant drop-out are outlined in the supplementary material. Eighty participants, consisting of 15 controls, 17 individuals with SCD, 34 with MCI, and 14 with AD dementia, were included for final analysis. Written informed consent was obtained from all participants prior to participation. This study was performed in accordance with the Declaration of Helsinki and the rules and regulations of the local institutional review boards, and approved by the medical ethics committee in Maastricht.

Clinical assessment

Memory clinic patients underwent standard clinical assessments, including regular neurological and psychiatric examinations and a complete neuropsychological assessment. Controls underwent a similar neuropsychological assessment. For all participants, educational level (8 level scale¹⁹), medical history and current medication were recorded, and an MMSE was administered²⁰. Raw scores on neuropsychological tests were converted to Z-scores, adjusted for age, sex, and education using normative data²¹⁻²⁴. Controls had no impairment in memory performance (Z-score > -1.5 standard deviation (SD)). Individuals were diagnosed with subjective cognitive decline (SCD) when self-experienced cognitive decline was reported in comparison with a previously normal status and unrelated to an acute event, but no objective cognitive impairment was detected on any of the neuropsychological tests¹⁶. Diagnosis of mild cognitive impairment (MCI) was made when concern about cognitive functioning was reported by the patient or an informant, cognitive impairment was detected on at least one cognitive test (Z-score < -1.5 SD), and dementia was absent¹⁷. AD (possible or probable) diagnosis was made when individuals met the NIA-AA core clinical criteria

for AD and neurodegeneration (i.e. mean MTA score ≥ 1.5) was evident¹⁸. Our study sample also contained individuals with a clinical diagnosis of AD but absence of neurodegeneration (n=5, 36% of AD cases). This group is referred henceforth as ‘other dementia’. Participant characteristics and Z-scores on cognitive tests according to diagnostic group (cognitively normal, subjective cognitive decline, MCI, AD, other dementia) are provided in the supplementary material (tables S1 and S2). Diagnostic groups were pooled according to the severity of cognitive impairment (normal cognition (NC), i.e. CN and SCD pooled, MCI and dementia (i.e. AD and other dementia)) for our statistical analyses, to reduce the number of group comparisons.

MR imaging

Images were acquired on a 3.0 Tesla MRI system with a 32-channel head coil (Philips, Achieva TX; Philips Healthcare, Best, the Netherlands). The imaging protocol was split into two sessions to reduce the burden of the long acquisition time and to minimize motion artifacts. During the first session, 3D T1-weighted gradient-echo (repetition time (TR) 8 ms; echo time (TE) 4 ms; flip angle 8°; 1 mm cubic voxel size) for anatomical reference, 3D T2-weighted FLAIR (TR/TE/inversion time (TI) of 4800/290/1650 ms; 1 mm cubic voxel size) for determination of WMH volume, and multisection T2-weighted turbo spin echo (TSE) and T2-weighted fast field echo (FFE) images with a section thickness of 5 mm in the transverse plane were acquired for detection of other tissue abnormalities (i.e. microbleeds, lacunar infarcts, hemorrhages and other infarcts). During the second session (median time between sessions 0 days, interquartile range 0-4 days) dual-time resolution DCE-MRI was acquired^{10, 12}. The dual-time DCE-MRI protocol had a short dynamic scan time (DST, 3.2 s) during the steep signal changes in initial circulations of the contrast agent and longer scan times (30.5 s) during the longer leakage phase with much slower signal changes. Before contrast administration, (precontrast) scans of both sequences were acquired. Subsequently, a bolus injection was performed during the fast sequence (0.1 mmol/kg gadobutrol, Gadavist®, Bayer AG, Leverkusen, Germany), intravenously in the antecubital vein (injection rate 3 mL/s, 20 mL saline flush). The fast sequence consisted of 29 volumes (TR/TE 5.3/2.5ms, voxel size 2 x 2 x 5 mm) and the slow sequence consisted of 35 volumes (TR/TE 5.6/2.5 ms, voxel size 1 x 1 x 2 mm). T1 mapping was performed prior to contrast administration and dynamic imaging to enable the conversion of the contrast-enhanced tissue signal intensities to concentrations²⁵.

Brain region segmentation

Automated software (FreeSurfer, version 5.1.0) was used with default settings as described previously²⁶. The segmentation was visually checked by one author (W.M.F.) and manual

adjustments were applied to correct for over- or under-estimation of the gray/white matter boundaries and to identify brain areas erroneously excluded during skull stripping. From the segmented brain regions, the estimated intracranial volume (ICV), overall gray matter volume (including cortical gray matter, deep gray matter (thalamus, caudate nucleus, putamen, pallidum, amygdala, and accumbens area), and hippocampus), hippocampal volume and total white matter volume (including WMH) were extracted ²⁶.

WMH volume was semi-automatically determined using an in-house developed, segmentation tool (GIANT) to differentiate between normal appearing white matter (NAWM) and WMH ²⁷. An algorithm was trained to classify WMH based on the FLAIR images. This was done by correcting the FLAIR scan for intensity non-uniformities ²⁸ and normalization of signal intensity²⁹. Seventeen scans from the current study with diverse WMH burden were randomly selected (scans from 2 control participants, 2 SCD, 10 MCI and 3 AD), and WMH were traced manually. Parameters for automated classification of WMH were derived based on the manually defined WMH regions. Subsequently, the actual WMH segmentation was performed by clicking on each identified WMH region in each section, providing a seed point to initiate a region growing algorithm. Manual corrections were performed where necessary by a single rater (I.V.) who was trained until excellent interrater variability was achieved (intra class correlation coefficient > 0.999) on an independent practice set of scans (n=20) that were also semi-automatically segmented by an experienced neuroradiologist. Total WMH volume was normalized to intracranial volume (ICV). WMH volume was first divided by ICV, then multiplied by a factor 10000, and subsequently log-transformed to better approximate a normal distribution.

Pharmacokinetic modeling and histogram analysis

BBB leakage rate was determined from pharmacokinetic model analysis in which the wash-in rate of gadolinium contrast agent was corrected for the vascular input. The vascular input function was extracted from manually selected voxels (n~20) within the sagittal sinus of each individual. Individual hematocrit levels were included in the model to better estimate the gadolinium concentration within voxels containing blood. Two-compartment pharmacokinetic modeling was applied for each voxel using the Patlak graphical approach to obtain the local BBB leakage rate (K_i in min^{-1} as the slope of the Patlak plot) ^{10, 12, 30}. As leakage was very subtle, noisy Patlak graphs were obtained, which resulted in voxels with positive as well as negative K_i values ^{12, 31}. To discern the measurable leakage from noise, a histogram analysis was applied to each investigated tissue region ¹². The histograms were naturally skewed to positive K_i values, and the noise was suppressed by subtracting the negative bins from the positive part ¹². The voxels with negative K_i values as well

as the positives that the negatives cancelled out were set to 0 (noise estimation), because in these voxels no significant leakage could be measured. Subsequently, the mean K_i of all voxels (including those that were set to 0) within each region was calculated. The BBB leakage volume fraction V_l was calculated as the percentage of voxels not included in the noise estimation out of the total number of voxels within a specified ROI.

Visual rating MRI markers of neurodegeneration and cerebrovascular damage

Several MRI markers of cerebrovascular damage and neurodegeneration were visually rated by an experienced neuroradiologist (W.M.P.) who was blinded to the clinical data. Medial temporal lobe atrophy (MTA) was rated bilaterally on coronal T1-weighted images using a previously described rating scale (range 0-4 for the average of left and right)³². WMH load was scored according to the Fazekas scale³³. Lacunes were identified on FLAIR and T2-weighted spin echo images³⁴. Cerebral microbleeds were identified on T2-weighted gradient echo images in cortical or subcortical regions³⁵. Old cortical infarcts of >15 mm in diameter were identified on T2-weighted spin echo and FLAIR images, and old cerebral hemorrhage was identified on T2-weighted gradient echo images.

Neuropsychological assessment

We focused on two cognitive outcomes in our analysis. Memory performance was evaluated because it is the primary cognitive domain that is affected in AD³⁶, and information processing speed was chosen because of its previously reported close association with vascular cognitive impairment, in particular WMH³⁷. Memory performance was assessed by means of the 15-word verbal learning task (VLT)²¹, and delayed memory recall (number of correctly recalled words after a 20-minute delay) (VLTdr) was used as outcome measure. The letter-digit substitution test (LDST) was used to measure information processing speed, with the number of correct items used as outcome measure²⁴. Additional neuropsychological tests that were assessed but only used for diagnostic purpose are outlined in the supplementary material (table 2).

Vascular risk factor assessment

Hypertension was defined as current use of antihypertensive medication and hypercholesterolemia was defined as current use of cholesterol lowering medication. Diabetes was defined as known history of diabetes type 1 or 2 and cardiovascular disease as known history of myocardial infarction, angina pectoris, and/or cardiac arrhythmia.

Laboratory measurements

Blood samples were collected and analyzed at Maastricht University Medical Center, to measure hematocrit levels and renal function for this study. Apolipoprotein E (ApoE) genotyping was performed using a method of polymerase chain reaction. Individuals with at least one $\epsilon 4$ allele were classified as ApoE4 positive.

Statistical analysis

To test for differences in group characteristics, one-way analyses of variance (ANOVA) with post-hoc Tukey pairwise comparisons were performed. Kruskal-Wallis tests with post-hoc Dunn's pairwise comparisons were applied to assess group differences for data with a skewed distribution because of unequal group sizes. Pearson chi-square or Fisher's exact test were used when appropriate to assess differences in proportions between groups for categorical variables. We ran regression models with BBB leakage as dependent variable and age as independent variable, corrected for pooled diagnostic group.

We set out to investigate the relationship between WMH volume and regional BBB integrity within the total gray and white matter and within their subregions (cortical gray matter, deep gray matter, hippocampus, NAWM and WMH) using multiple linear regression analyses. Normalized and log-transformed WMH volume was included as dependent variable, and regional leakage rate or volume (both cuberoot-transformed to better approximate a normal distribution, see supplemental figure 3) as independent variable, corrected for age and pooled diagnostic group. Post-hoc, we added the interaction term between pooled diagnostic group and the leakage measures to assess whether the associations were different between the diagnostic groups.

Because previous work identified increased BBB disruption within the hippocampus in individuals with MCI ⁹, we examined the association between HV and BBB leakage within the hippocampus. We applied multiple linear regression with HV (corrected for intracranial volume and log-transformed to better approximate a normal distribution) as dependent variable, and hippocampal BBB leakage (cuberoot-transformed) as independent variable, with age and pooled diagnostic group as covariates. Afterwards, the interaction term between pooled diagnostic group and leakage was included to examine whether the associations were similar for the diagnostic groups.

Two mediation models were run to test for direct and indirect associations between cognition and BBB leakage. The first model included memory performance as dependent variable, hippocampal BBB leakage as independent variable, and HV as mediator variable. The second model included information processing speed performance as dependent variable, white matter

BBB leakage as independent variable, and WMH volume as mediator variable (see supplemental data for details)³⁸.

Due to the exploratory nature of this study we did not correct for multiple comparisons in our main analyses. A threshold of $\alpha < 0.05$ was used to determine statistical significance. All p-values are two-tailed. All analyses were conducted using R statistical software (version 3.3.3)³⁹.

RESULTS

Participant characteristics

The group characteristics of the participants according to pooled diagnostic group are summarized in table 1. Individuals with MCI had lower scores on the MMSE, VLTdr, and LDST tests compared with the cognitively normal individuals. Individuals with dementia had lower scores on the MMSE and VLTdr, smaller HV, and higher MTA scores compared with the MCI and NC groups, and lower scores on the LDST test compared with the NC group. There were no group differences in leakage rate or leakage volume in any of the examined brain regions (supplemental table S3a and S3b), and leakage rate and leakage volume were not associated with age after correcting for pooled diagnostic group (data not shown).

WMH volume and BBB leakage

The associations between WMH volume and regional BBB leakage are provided in table 2. In the total studied sample, WMH volume was positively associated with BBB leakage rate within the total gray and white matter (Multiple $R^2 > 0.31$) (figure 1). This association remained significant for BBB leakage within the cortex, deep gray matter, NAWM and WMH, but not within the hippocampus. The results were similar for the association between WMH volume and leakage volume. There were no significant interaction effects between leakage and pooled diagnostic group (table S4).

Figure 1: White matter hyperintensity volume versus blood-brain barrier leakage[§]

[§]BBB leakage volume was cuberoot transformed, WMH volume was normalized to ICV and log-transformed.

Fit and 95% confidence interval of ordinary least squares regression model adjusted for age and pooled diagnosis is shown.

HV and BBB leakage

HV was not significantly associated with BBB leakage within the hippocampus in the total sample (table 3 and figure S4), and there was no interaction effect between leakage and pooled diagnostic group (table S5).

Cognitive performance and BBB leakage

Results of the mediation model analyses of BBB disruption on cognition are presented in figure 2, table S6a, and table S6b. There were no direct or indirect effects between hippocampal leakage rate/volume and memory performance, and between leakage volume and information processing speed. In contrast, a direct effect was present between white matter leakage rate and information processing speed. When WMH volume was added to the model, the mediating (i.e., indirect) effect of WMH volume was significant for the relationship between leakage rate and information processing speed (B [95% confidence interval] = -222.48 [-540.81; -27.10]), while the direct effect between leakage rate and information processing speed disappeared. There were no significant interaction effects between BBB leakage rate or leakage volume and pooled diagnostic group on cognition (Table S7).

Table 1: Group characteristics according to pooled diagnosis

	NC (n=32)	MCI (n=34)	Dementia (n=14)	p-value
<u>Participant characteristics</u>				
Age (years), mean (SD)	71.8 (7.0)	68.6 (6.3)	71.6 (6.7)	0.130
Male, n (%)	21 (65.6)	19 (55.9)	11 (78.6)	0.318
Education (level) [§]	5.0 [5.0;6.0]	5.0 [4.0;6.0]	5.0 [4.0;6.0]	0.267
eGFR (mL/min)	75.0 [69.6;82.7]	81.0 [71.9;89.2]	74.0 [69.8;81.3]	0.237
ApoE4 positive, n (%) [*]	13 (41.9)	12 (36.4)	8 (57.1)	0.419
Hypertension, n (%)	13 (40.6)	13 (38.2)	6 (42.9)	0.953
Hypercholesterolemia, n (%)	10 (31.2)	7 (20.6)	4 (28.6)	0.599
Diabetes Mellitus, n (%)	1 (3.12)	5 (14.7)	2 (14.3)	0.216
Cardiovascular disease, n (%)	10 (31.2)	11 (32.4)	3 (21.4)	0.769
Hematocrit	0.45 [0.42;0.46]	0.43 [0.42;0.46]	0.44 [0.41;0.48]	0.452
<u>Imaging characteristics</u>				
HV (%ICV)	0.25 [0.20;0.30]	0.22 [0.20;0.26]	0.18 [0.17;0.21] ^{ce}	0.001
WMH volume (%ICV)	0.26 [0.18;0.31]	0.34 [0.20;0.55]	0.30 [0.15;0.47]	0.392
LI present, n (%)	3 (9.4)	6 (17.6)	3 (21.4)	0.495
CMB presence, n (%)	1 (3.12)	6 (17.6)	3 (21.4)	0.078
IS present, n (%)	1 (3.12)	6 (17.6)	0 (0.00)	0.075
ICH present, n (%)	0 (0.00)	1 (2.94)	0 (0.00)	1.000
Fazekas score	1.00[1.00;2.00]	1.00[1.00;2.00]	1.00[1.00;1.75]	0.861
Mean MTA	0.50 [0.00;1.00]	0.75 [0.00;1.38]	1.50 [0.62;2.38] ^{bd}	0.010
Contrast agent (ml), mean (SD)	7.6 (1.2)	7.5 (1.3)	7.6 (1.3)	0.970
<u>Cognitive measures</u>				
MMSE [*]	29.0 [28.0;30.0]	27.0 [26.0;29.0] ^a	24.5 [21.0;27.0] ^{ce}	<0.001
VLTdr score, mean (SD) [*]	9.19 (2.85)	5.74 (3.76) ^c	2.69 (1.25) ^{cd}	<0.001
LDST score, mean (SD) [*]	43.87 (10.30)	34.79 (8.73) ^b	31.80 (12.45) ^b	<0.001

Abbreviations: NC, normal cognition; SD, standard deviation; eGFR, estimated glomerular filtration rate; HV, hippocampal volume; ICV, intracranial volume; LI, lacunar infarct; CMB, cerebral microbleed; IS, ischemic stroke; ICH, intracerebral hemorrhage; MTA, medial temporal lobe atrophy; MMSE, mini-mental state examination; VLTdr, verbal learning task delayed recall; LDST, letter-digit substitution test. Values represent median [interquartile range] unless otherwise specified. Significant association are depicted in bold.

^ap<0.05 vs NC, ^bp<0.01 vs NC, ^cp<0.001 vs NC, ^dp<0.05 vs MCI, ^ep<0.01 vs MCI;

[§]Education level was based on an 8-level scale¹⁹

^{*}Data were missing; MMSE, n=2; ApoE4, n=1; VLTdr, n=1; LDST, n=5

Table 2: Associations between WMH volume and the regional leakage measures leakage rate and leakage volume[§]

	<u>Leakage rate</u>			<u>Leakage volume</u>		
	K _i	95% CI	p-value	V _i	95% CI	p-value
Total GM	.22	[0.03;0.41]	0.029	.23	[0.03;0.42]	0.023
Total WM	.25	[0.06;0.44]	0.010	.24	[0.05;0.43]	0.014
Cortex	.21	[0.01;0.40]	0.039	.22	[0.02;0.41]	0.030
Deep GM	.22	[0.03;0.40]	0.028	.22	[0.03;0.41]	0.028
Hippocampus	.12	[-0.07;0.32]	0.223	.11	[-0.08;0.31]	0.264
NAWM	.25	[0.06;0.44]	0.011	.24	[0.05;0.43]	0.016
WMH	.25	[0.07;0.44]	0.010	.27	[0.08;0.45]	0.006

NOTE: Values represent standardized beta coefficients. Significant associations (i.e. $p < 0.05$) are depicted in bold. §BBB leakage rate and leakage volume were cuberoot transformed and WMH volume was normalized to ICV and log transformed for the analyses. Analyses were adjusted for age and pooled diagnosis.

Abbreviations: MCI, mild cognitive impairment; GM, gray matter; WM, white matter; NAWM, normal appearing white matter; WMH, white matter hyperintensities; CI, confidence interval.

Table 3: Associations between hippocampal volume and leakage rate or leakage volume within the hippocampus[§]

	<u>Leakage rate</u>			<u>Leakage volume</u>		
	K _i	95% CI	p-value	V _i	95% CI	p-value
Hippocampus	.14	[-0.05;0.33]	0.14	.13	[-0.06;0.32]	0.18

NOTE: Values represent standardized beta coefficients.

§BBB leakage rate and leakage volume were cuberoot-transformed and HV was normalized to ICV and log-transformed for the analyses. Analyses were adjusted for age and pooled diagnosis.

Abbreviations: MCI, mild cognitive impairment; GM, gray matter; WM, white matter; NAWM, normal appearing white matter; WMH, white matter hyperintensities; CI, confidence interval.

Figure 2: Overview of mediation models with standardized path strengths

The arrow paths show standardized beta values derived from the mediation analysis. The upper model shows a significant (*) negative relationship between white matter (WM) BBB leakage rate (K_i) and information processing speed (c path). This direct association is no longer significant when WMH volume is included as a mediator in the model (c' path), but the association now indirectly operates through WMH volume (path a and path b). None of the associations between hippocampal leakage, hippocampal volume (HV), and memory performance were significant (lower model). When BBB disruption was assessed in the form of leakage rate (V_i), none of the pathways were significant (see table S6a and S6b). Analyses were corrected for age (at MRI), sex, educational level, time between MRI and neuropsychological assessment, and pooled diagnostic group.

DISCUSSION

Present findings

The aim of this study was to examine contributions of BBB disruption to the interplay of cSVD, neurodegeneration, and cognitive performance in a study sample of individuals within the range from normal cognition to dementia. Our findings demonstrate that BBB leakage represents a key process that is associated with cognitive impairment in individuals with cSVD across the AD spectrum. While previous studies have already revealed BBB leakage in clinical populations with cSVD and AD, we now extend these findings by demonstrating that (i) BBB disruption is associated with cSVD throughout the whole brain, independent of disease state, (ii) BBB disruption is associated with impaired cognitive performance: information processing speed appears to be affected by white matter BBB leakage in an indirect way, through mediation of WMH, and (iii) local BBB disruption is not related to hippocampal volume reduction, neither in cognitively normal controls nor in memory clinic patients. As such, in this population of patients, most of

whom had cognitive impairment or dementia, BBB disruption may form an indirect pathophysiologic pathway to cognitive decline.

Associations between WMH volume and BBB leakage

We observed that global BBB leakage within the total gray and total white matter was positively associated with WMH volume. When we detailed this examination to subregions, we found that this association was apparent within the cortex, deep gray matter, NAWM and WMH, but not within the hippocampus. Our findings are consistent with previous studies demonstrating positive associations between BBB leakage and cSVD using DCE-MRI. For example, when cSVD patients were compared to controls without neurological disease, higher BBB leakage rates were found in the NAWM⁴⁰ and total white matter⁴¹ of the cSVD patient groups. Other studies showed larger leakage volumes within the NAWM, WMH, and cortex in cSVD patients compared to age-matched controls¹⁰, and higher BBB leakage within the white matter of cSVD patients compared with cortical stroke patients¹¹. Also, two recent studies in a cohort of lacunar and cortical stroke patients with a larger sample size ($n > 200$) and age range (34-90 and 34-97 years) demonstrated a positive association between WMH severity and BBB leakage within the deep gray matter, NAWM and WMH^{14,42} and by proximity to WMH edge. Our results now showed similar positive associations between WMH volume and BBB leakage within gray and white matter and thus further support the idea that cSVD is a dynamic and progressive whole brain disease⁴³. More importantly, our findings extend the present literature by showing that the association between BBB disruption and cSVD and cognition is independent of cognitive disease stage, and can be observed in individuals across the spectrum from normal cognitive aging to Alzheimer-type dementia. This suggests that BBB leakage plays a constant role in pathological cSVD processes across all disease states.

Associations between HV and BBB leakage

Hippocampal neurodegeneration is a core feature of AD, but it is also associated with cerebrovascular brain damage^{44,45}. Previous work has shown that increases in WMH volume are related to smaller hippocampal volumes in the presence of AD pathology (abnormal cerebral amyloid- β levels)⁴⁶. Furthermore, previous work has demonstrated increased BBB leakage rate within the hippocampus in individuals with MCI compared with age-matched cognitively normal controls. Therefore, we expected that hippocampal BBB leakage would be negatively associated with HV in the present study sample. Surprisingly, we did not find a negative association between hippocampal leakage and HV, which suggests that hippocampal volume loss may not be associated with BBB disruption in a direct way. BBB leakage might occur at an earlier stage in the development

of hippocampal loss, perhaps explaining the difference between the present and previous work which included individuals with less cognitive impairment than in the present study⁹. Alternatively, it could well be that BBB leakage is more strongly associated with vascular pathology than AD pathology, and that our results were driven by the large proportion of individuals with vascular cognitive impairment, who may have less hippocampal atrophy compared with individuals with cognitive impairment due to AD. Unfortunately, the amyloid- β status of the participants in the present study is unknown. Furthermore, the cross-sectional nature of our study might have been insensitive to the detection of associations between leakage and hippocampal neurodegeneration, for which a longitudinal design might elucidate different insights.

BBB disruption and disease state

Recently, the association between BBB integrity and AD has been topic of debate, attesting to the increasing awareness for the hypothesis that an interaction between vascular and AD pathology may play a role in the onset or progression of AD^{46,47}. Two recent studies that applied DCE-MRI in cognitively normal individuals and patients with MCI and AD found elevated BBB leakage in patients compared with cognitively normal controls, and with increasing age^{9,12}. These studies underline the importance of microvascular disruption in the AD cascade of pathological events. However, only one of these studies corrected for some markers of cerebrovascular damage in their analyses, but the vascular pathology in this study sample was generally low¹². It remains unclear, however, whether the increased BBB disruption with increasing age and MCI status observed in the other study was actually driven by increased cSVD pathology in patients compared with the controls. In our study sample, we were not able to identify differences in BBB leakage between individuals with normal cognition, MCI or AD in any of the examined regions, which is likely due to the relatively high and equal vascular burden between our diagnostic groups. Moreover, we did not find any associations between age and BBB disruption, though age was associated with increased BBB leak measured in several ways in a previous systematic review⁴⁸. A limited age range and sample size could have masked possible associations, and our observations are in agreement with other studies that also report absence of a BBB disruption-age association⁴⁰⁻⁴².

BBB disruption and cognition

To examine the clinical relevance of BBB disruption, we tested for associations between BBB leakage and cognitive performance. While there seems to be no direct effect of BBB disruption on information processing speed after correcting for white matter hyperintensity volume, we did identify an indirect association between white matter BBB leakage rate and information processing

speed that was mediated by WMH volume over the entire study population. Apart from two previous studies reporting a negative relationship between BBB leakage and global cognition (i.e. MMSE and Addenbrooke's Cognitive Examination Revised score)^{12, 14}, this is the first study reporting associations between specific cognitive domains and BBB disruption. Our findings are in line with previous work that suggests that BBB disruption in NAWM may eventually lead to cSVD pathology⁴². It can be hypothesized that long-lasting subtle BBB disruption throughout the brain is reflected by the amount of WMH burden, and WMH burden in turn reflects white matter damage which is associated with decreases in processing speed^{49, 50}. While decreases in processing speed are not a typical feature of AD, like decline in episodic memory, previous work has suggested that processing speed is an important component of cognition that influences performance on other cognitive domains⁵¹. As such, BBB leakage could indirectly affect many cognitive domains through its effects on information processing speed.

Study considerations

Strengths of this study include the availability of BBB leakage data for most of the cerebrum, which allowed us to measure leakage markers within the total gray and white matter and various subregions. The inclusion of individuals from various disease stages allowed for substantial variation in cerebrovascular damage load, neurodegeneration, and cognition. This study also has limitations. Measurement of BBB leakage within smaller regions of interest such as the hippocampus are more prone to measurement error, and partial volume effects of signal from structures such as the choroid plexus or meninges cannot be ruled out. Furthermore, our total sample was relatively small, and our AD sample was smaller compared to the NC and MCI groups and contained individuals without evidence of neurodegeneration, although 80% of these individuals with low MTA scores carried at least one ApoE ϵ 4 allele. Another limitation of this study is that AD biomarkers amyloid-beta and tau were unknown, and as such we were not able to assess associations between BBB leakage and these variables or to control for them in our analyses. In addition, we might have underestimated the number of individuals with hypertension since some individuals may not receive medication for this, and the set of scans used to train the rater for WMH scoring was relatively small (n=20, but high inter-rater reliability was achieved). Although mediation implies a causal process, our data are of cross-sectional nature and we are unable to conclude whether the associations are cause-effect or not. Future studies are needed to determine the associations between BBB disruption and AD biomarkers (amyloid-beta and tau) while accounting for the effects of cerebrovascular pathology. Longitudinal studies could possibly shed more light on time evolution of BBB disruption in the context of cSVD, neurodegeneration, and

cognitive decline. Our findings suggest that the development of treatment strategies that improve cerebrovascular health, in particular by enhancing BBB integrity, may slow down deterioration in information processing speed not only in cSVD patients, but also in individuals with AD.

CONCLUSION

This study shows that BBB disruption is related to cSVD severity throughout the whole brain in a cross-sectional study sample of older individuals ranging from normal cognition to Alzheimer-type dementia. BBB disruption negatively affects information processing speed performance through WMH pathology.

REFERENCES

1. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *The Lancet Neurology* 2013;12:207-216.
2. Arvanitakis Z, Capuano AW, Leurgans SE, Bennett DA, Schneider JA. Relation of cerebral vessel disease to Alzheimer's disease dementia and cognitive function in elderly people: a cross-sectional study. *The Lancet Neurology* 2016;15:934-943.
3. Thal DR, Ghebremedhin E, Orantes M, Wiestler OD. Vascular pathology in Alzheimer disease: correlation of cerebral amyloid angiopathy and arteriosclerosis/lipohyalinosis with cognitive decline. *J Neuropathol Exp Neurol* 2003;62:1287-1301.
4. Kester MI, Goos JD, Teunissen CE, et al. Associations between cerebral small-vessel disease and Alzheimer disease pathology as measured by cerebrospinal fluid biomarkers. *JAMA neurology* 2014;71:855-862.
5. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke* 2011;42:2672-2713.
6. Yamazaki Y, Kanekiyo T. Blood-Brain Barrier Dysfunction and the Pathogenesis of Alzheimer's Disease. *International journal of molecular sciences* 2017;18.
7. Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 2008;57:178-201.
8. Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nature reviews Neuroscience* 2004;5:347-360.
9. Montagne A, Barnes SR, Sweeney MD, et al. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* 2015;85:296-302.
10. Zhang CE, Wong SM, van de Haar HJ, et al. Blood-brain barrier leakage is more widespread in patients with cerebral small vessel disease. *Neurology* 2017;88:426-432.
11. Wardlaw JM, Doubal F, Armitage P, et al. Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. *Annals of neurology* 2009;65:194-202.
12. van de Haar HJ, Burgmans S, Jansen JF, et al. Blood-Brain Barrier Leakage in Patients with Early Alzheimer Disease. *Radiology* 2016;152244.
13. Wardlaw JM. Blood-brain barrier and cerebral small vessel disease. *J Neurol Sci* 2010;299:66-71.
14. Wardlaw JM, Makin SJ, Valdés Hernández MC, et al. Blood-brain barrier failure as a core mechanism in cerebral small vessel disease and dementia: evidence from a cohort study. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2017;13:634-643.
15. Iturria-Medina Y, Sotero RC, Toussaint PJ, Mateos-Perez JM, Evans AC. Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. *Nat Commun* 2016;7:11934.
16. Jessen F, Amariglio RE, van Boxtel M, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2014;10:844-852.
17. Petersen RC. Mild cognitive impairment as a diagnostic entity. *Journal of internal medicine* 2004;256:183-194.
18. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2011;7:263-269.
19. De Bie S. Standaardvragen 1987: Voorstellen voor uniformering van vraagstellingen naar achtergrondkenmerken en interviews [Standard questions 1987: Proposal for uniformization of questions regarding background variables and interviews]. Leiden: Leiden University Press 1987.
20. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research* 1975;12:189-198.
21. Van der Elst W, van Boxtel MP, van Breukelen GJ, Jolles J. Rey's verbal learning test: normative data for 1855 healthy participants aged 24-81 years and the influence of age, sex, education, and mode of presentation. *Journal of the International Neuropsychological Society : JINS* 2005;11:290-302.

22. Van der Elst W, Van Boxtel MP, Van Breukelen GJ, Jolles J. The Stroop color-word test: influence of age, sex, and education; and normative data for a large sample across the adult age range. *Assessment* 2006;13:62-79.
23. Van der Elst W, Van Boxtel MP, Van Breukelen GJ, Jolles J. The Concept Shifting Test: adult normative data. *Psychological assessment* 2006;18:424-432.
24. van der Elst W, van Boxtel MP, van Breukelen GJ, Jolles J. The Letter Digit Substitution Test: normative data for 1,858 healthy participants aged 24-81 from the Maastricht Aging Study (MAAS): influence of age, education, and sex. *Journal of clinical and experimental neuropsychology* 2006;28:998-1009.
25. Larsson HB, Courivaud F, Rostrup E, Hansen AE. Measurement of brain perfusion, blood volume, and blood-brain barrier permeability, using dynamic contrast-enhanced T(1)-weighted MRI at 3 tesla. *Magn Reson Med* 2009;62:1270-1281.
26. Fischl B. FreeSurfer. *NeuroImage* 2012;62:774-781.
27. Jacobs HI, Clerx L, Gronenschild EH, Aalten P, Verhey FR. White matter hyperintensities are positively associated with cortical thickness in Alzheimer's disease. *Journal of Alzheimer's disease : JAD* 2014;39:409-422.
28. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE transactions on medical imaging* 1998;17:87-97.
29. Nyul LG, Udupa JK. On standardizing the MR image intensity scale. *Magn Reson Med* 1999;42:1072-1081.
30. Patlak CS, Blasberg RG, Fenstermacher JD. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. *J Cereb Blood Flow Metab* 1983;3:1-7.
31. van de Haar HJ, Jansen JFA, Jeukens C, et al. Subtle blood-brain barrier leakage rate and spatial extent: Considerations for dynamic contrast-enhanced MRI. *Med Phys* 2017;44:4112-4125.
32. Scheltens P, Leys D, Barkhof F, et al. Atrophy of medial temporal lobes on MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. *Journal of neurology, neurosurgery, and psychiatry* 1992;55:967-972.
33. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol* 1987;149:351-356.
34. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *The Lancet Neurology* 2013;12:822-838.
35. Greenberg SM, Vernooij MW, Cordonnier C, et al. Cerebral microbleeds: a guide to detection and interpretation. *The Lancet Neurology* 2009;8:165-174.
36. Jahn H. Memory loss in Alzheimer's disease. *Dialogues in clinical neuroscience* 2013;15:445-454.
37. Wiseman SJ, Booth T, Ritchie SJ, et al. Cognitive abilities, brain white matter hyperintensity volume, and structural network connectivity in older age. *Human brain mapping* 2018;39:622-632.
38. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *Journal of personality and social psychology* 1986;51:1173-1182.
39. R Core Team. *R: A Language and Environment for Statistical Computing* 2016.
40. Topakian R, Barrick TR, Howe FA, Markus HS. Blood-brain barrier permeability is increased in normal-appearing white matter in patients with lacunar stroke and leucoaraiosis. *Journal of neurology, neurosurgery, and psychiatry* 2010;81:192-197.
41. Taheri S, Gasparovic C, Huisa BN, et al. Blood-brain barrier permeability abnormalities in vascular cognitive impairment. *Stroke* 2011;42:2158-2163.
42. Munoz Maniega S, Chappell FM, Valdes Hernandez MC, et al. Integrity of normal-appearing white matter: Influence of age, visible lesion burden and hypertension in patients with small-vessel disease. *J Cereb Blood Flow Metab* 2017;37:644-656.
43. Shi Y, Wardlaw JM. Update on cerebral small vessel disease: a dynamic whole-brain disease. *Stroke and vascular neurology* 2016;1:83-92.
44. Kim GH, Lee JH, Seo SW, et al. Hippocampal volume and shape in pure subcortical vascular dementia. *Neurobiology of aging* 2015;36:485-491.
45. Guzman VA, Carmichael OT, Schwarz C, Tosto G, Zimmerman ME, Brickman AM. White matter hyperintensities and amyloid are independently associated with entorhinal cortex volume among

- individuals with mild cognitive impairment. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2013;9:S124-131.
46. Freeze WM, Jacobs HI, Gronenschild EH, et al. White Matter Hyperintensities Potentiate Hippocampal Volume Reduction in Non-Demented Older Individuals with Abnormal Amyloid-beta. *Journal of Alzheimer's disease : JAD* 2017;55:333-342.
 47. Iadecola C. The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease. *Neuron* 2017;96:17-42.
 48. Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease--systematic review and meta-analysis. *Neurobiology of aging* 2009;30:337-352.
 49. Baykara E, Gesierich B, Adam R, et al. A Novel Imaging Marker for Small Vessel Disease Based on Skeletonization of White Matter Tracts and Diffusion Histograms. *Annals of neurology* 2016;80:581-592.
 50. Burns JM, Church JA, Johnson DK, et al. White matter lesions are prevalent but differentially related with cognition in aging and early Alzheimer disease. *Arch Neurol* 2005;62:1870-1876.
 51. Salthouse TA. Shared and unique influences on age-related cognitive change. *Neuropsychology* 2017;31:11-19.
 52. Stroop JR. Studies of interference in serial verbal reactions. *J Exp Psychol* 1935;18(6):643-662.
 53. Efron B. Better Bootstrap Confidence Intervals. *Journal of the American Statistical Association* 1987;82:171-185.
 54. Williams J, MacKinnon DP. Resampling and Distribution of the Product Methods for Testing Indirect Effects in Complex Models. *Structural equation modeling : a multidisciplinary journal* 2008;15:23-51.
 55. Carpenter J, Bithell J. Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians. *Statistics in medicine* 2000;19:1141-1164.

SUPPLEMENTARY MATERIAL

Supplementary methods

Exclusion criteria

Exclusion criteria for all participants included impaired renal function (as determined by estimated glomerular filtration rate (eGFR) <30 mL/min), and presence of psychiatric or neurological disorders other than AD, cerebral small vessel disease, or non-invalidating stroke, that might be related to the cognitive impairment (including major depression (less than 12 months before inclusion), history of schizophrenia, bipolar disorder, psychotic disorder NOS or treatment for a psychotic disorder, cognitive impairment due to alcohol abuse, epilepsy, Parkinson's disease, MS, brain surgery, brain trauma, electroshock therapy, kidney dialysis, Meniere's disease and brain infections). Participants with recent (e.g. <3 months before inclusion) ischemic or hemorrhagic stroke were excluded.

Participant drop-out

In total, 101 participants were enrolled in this study. Two control participants and six patients were excluded because of low renal function. One patient dropped out of the study voluntarily after the first MRI. Two patients with structural abnormalities (meningioma, lipoma) were excluded. DCE-MRI data for one control participant and two patients could not be processed because of premature scan termination. Two patients were excluded because of severe motion artifacts on DCE-MRI. One control participant and two patients were excluded because the brain region segmentation failed after multiple attempts. Two participants were diagnosed with other dementia types (frontotemporal dementia and 'unknown') after participation in the study and were excluded (Figure S1).

Additional neuropsychological assessment

Additional tests used for neuropsychological assessment included memory performance measured as immediate (sum of the number of correctly recalled words directly after each of the 5 trials) memory recall according to the 15-word verbal learning task (VLT).²¹ Attention was measured using the average of the Stroop Color Word Test (SCWT) cards 1 and 2^{22,52}, and using the average of the concept shifting test (CST) part A and B²³. Executive functioning was measured using the interference index from the SCWT and the interference index from the CST^{22,23,52}. These tests measured two components of executive functioning, sensitivity to interference and concept shifting

respectively. The median (interquartile range) time between neuropsychological assessment and MRI acquisition was 3 [0-8] months.

Figure S1: Participant drop-out process

Mediation model analysis

For the first model, the total association between memory performance (uncorrected delayed memory recall score) as dependent variable and hippocampal BBB leakage as independent variable was assessed (see figure S2A, path c). Then we tested whether HV mediated this relationship by testing two associations: 1) the association between HV as dependent variable, and hippocampal BBB leakage as independent variable (figure S2B, path a), and 2) the association between memory performance as dependent variable, and HV (figure S1B, path b) and hippocampal BBB leakage (figure S1B, path c') as independent variables. In the second model we used information processing speed performance (uncorrected LDST score) as dependent variable, leakage within the total white matter as independent variable, and WMH volume as mediator variable. All analyses were corrected for age (at MRI), sex, educational level, time between MRI and neuropsychological assessment, and pooled diagnostic group. When statistically significant associations were present in both path a and

path b, we tested the indirect mediating effect ($a*b$), or in other words: the extent to which the relationship between BBB disruption and cognitive functioning operates statistically through WMH volume or HV. The standard error parameters of the mediation models were bootstrapped, and the 95% confidence interval for each regression coefficient was calculated using the bias corrected and accelerated bootstrap method⁵³, which corrects for bias and skewness in the distribution of bootstrap estimates. The number of bootstrap samples in each analysis was set to 5000. Because the sampling distribution of the indirect effect $a*b$ is highly skewed, and the standard Z-method presumes a symmetric distribution, we report the bias corrected and accelerated 95% confidence interval of any indirect effects and assume the effect is significant if the confidence interval does not contain 0⁵⁴. Post-hoc, we ran multiple regression models with cognitive performance as dependent variable and the interaction term between pooled diagnostic group and leakage as independent variable to test for group differences in (interaction) slopes. Analyses were corrected for the main effect of leakage, the main effect of pooled diagnostic group, age (at MRI), sex, educational level, time between MRI and neuropsychological assessment, and normalized and log-transformed WMH volume or normalized and log-transformed HV. Because the sample sizes within each subgroup were small, non-parametric bootstrapping of the regression coefficients and bias-corrected and accelerated estimation of the 95% confidence intervals were performed to ensure valid coefficient estimation⁵⁵.

Figure S2: schematic model of mediation analysis

Figure S3: QQ plot and histogram of leakage rate within the cortex before and after cuberoot transformation

Figure S4: Hippocampal volume versus blood-brain barrier leakage[§]

[§]BBB leakage rate was cuberoot-transformed, HV was normalized to ICV and log-transformed

Fit and 95% confidence interval of ordinary least squares regression model is shown, corrected for age and pooled diagnosis. The slope of the regression was not significantly different from 0.

Table S1: Participant characteristics according to diagnostic group

	<u>Controls</u> <u>(N=15)</u>	<u>SCD</u> <u>(N=17)</u>	<u>MCI (N=34)</u>	<u>AD (N=9)</u>	<u>Other</u> <u>dementia</u> <u>(N=5)</u>	<u>P-value</u>
<u>Participant characteristics</u>						
Age (years)	71.9 (7.32)	71.7 (6.96)	68.6 (6.26)	72.4 (6.50)	70.0 (7.48)	0.348
Male, n(%)	10 (66.7%)	11 (64.7%)	19 (55.9%)	7 (77.8%)	4 (80.0%)	0.737
Education, median(IQR)	6.00 [5.50;6.00]	5.00 [4.00;5.00]	5.00 [4.00;6.00]	5.00 [4.00;6.00]	6.00 [5.00;6.00]	0.084
eGFR (mL/min), median(IQR)	71.1 [68.3;79.5]	77.5 [71.8;85.6]	81.0 [71.9;89.2]	70.5 [68.2;83.0]	74.7 [73.4;76.2]	0.164
MMSE, median(IQR)*	29.0 [29.0;30.0]	29.0 [27.0;29.0]	27.0 ^a [26.0;29.0]	22.5 ^{ace} [21.0;25.5]	26.5 ^a [24.8;27.2]	<0.001
ApoE4 positive, n(%)*	3 (20.0%)	11 (64.7%)	12 (36.4%)	4 (44.4%)	4 (80.0%)	0.041
Hypertension, n(%)	5 (33.3%)	8 (47.1%)	13 (38.2%)	5 (55.6%)	1 (20.0%)	0.682
Hypercholesterolemia, n(%)	5 (33.3%)	5 (29.4%)	7 (20.6%)	4 (44.4%)	0 (0.00%)	0.380
Diabetes Mellitus, n(%)	0 (0.00%)	1 (5.88%)	5 (14.7%)	2 (22.2%)	0 (0.00%)	0.317
Cardiovascular disease, n(%)	6 (40.0%)	4 (23.5%)	11 (32.4%)	3 (33.3%)	0 (0.00%)	0.562
Hematocrit (L/L)	0.45 [0.43;0.48]	0.44 [0.42;0.45]	0.43 [0.42;0.46]	0.45 [0.42;0.46]	0.41 [0.41;0.48]	0.647
<u>Imaging characteristics</u>						
HV (%ICV), median(IQR)	0.26 [0.22;0.31]	0.22 [0.20;0.29]	0.22 [0.20;0.26]	0.17 ^{abd} [0.16;0.19]	0.22 [0.19;0.22]	0.001
WMH volume (%ICV), median(IQR)	0.27 [0.21;0.33]	0.22 [0.17;0.31]	0.34 [0.20;0.55]	0.40 [0.26;0.74]	0.12 [0.11;0.28]	0.189
LI present, n(%)	3 (20.0%)	0 (0.00%)	6 (17.6%)	2 (22.2%)	1 (20.0%)	0.237
CMB presence, n(%)	0 (0.00%)	1 (5.88%)	6 (17.6%)	2 (22.2%)	1 (20.0%)	0.225

	Controls (N=15)	SCD (N=17)	MCI (N=34)	AD (N=9)	Other dementia (N=5)	P-value
IS present, n(%)	0 (0.00%)	1 (5.88%)	6 (17.6%)	0 (0.00%)	0 (0.00%)	0.310
ICH present, n(%)	0 (0.00%)	0 (0.00%)	1 (2.94%)	0 (0.00%)	0 (0.00%)	1.000
Fazekas score	1[1;2]	1[1;2]	1[1;2]	1[1;2]	1[1;1]	0.919
Mean MTA, median(IQR)	0.50 [0.00;1.00]	0.50 [0.00;0.50]	0.75 [0.00;1.38]	2.00 ^{acc} [1.50;2.50]	0.00 ^f [0.00;0.50]	<0.001
Contrast agent (ml)	7.23 (0.75)	7.85 (1.45)	7.49 (1.32)	7.73 (1.29)	7.24 (1.28)	0.656

Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; AD, Alzheimer type dementia; SD, standard deviation; eGFR, estimated glomerular filtration rate; L, liter; HV, hippocampal volume; ICV, intracranial volume; LI, lacunar infarct; CMB, cerebral microbleed; IS, ischemic stroke; ICH, intracerebral hemorrhage; MTA, medial temporal lobe atrophy; MMSE, mini-mental state examination; Values represent median [interquartile range] unless otherwise specified. *Data were missing; ApoE4, n=1

^ap<0.01 vs controls; ^bp<0.05 vs SCD; ^cp<0.01 vs SCD; ^dp<0.05 vs MCI; ^ep<0.01 vs MCI; ^fp<0.01 vs AD with biomarker evidence

Table S2: Z-scores neuropsychological tests for each diagnostic group

	<u>Z-score</u>			
	NC (n=32)	MCI (n=34)	Dementia (n=13) [§]	p-value
VLT	0.64 (1.15)	-0.73 ^c (1.22)	-1.67 ^c (1.43)	<0.001
VLT delayed	0.44 (1.12)	-1.08 ^c (1.41)	-2.07 ^{cd} (0.81)	<0.001
LDST [*]	0.54 (0.91)	-0.39 ^c (0.78)	-0.53 ^b (1.08)	<0.001
Stroop 1+2 [*]	-0.08 [-0.41;0.52]	-1.36 ^c [-1.98;-0.67]	-1.51 ^b [-2.25;-0.56]	<0.001
CST 1+2 [*]	0.46 [-0.10;0.97]	-0.54 ^a [-1.62;0.43]	-0.54 ^a [-1.87;0.31]	0.007
Stroop interference [*]	0.84 [-0.10;1.27]	-0.35 ^b [-0.89;0.19]	-0.69 ^b [-3.47;0.21]	<0.001
CST interference [*]	0.41 [-0.40;0.86]	-0.85 [-1.90;0.77]	-2.74 ^{bd} [-3.50;-2.33]	0.001

Values represent mean(standard deviation) or median[interquartile range]; ^ap<0.05 vs NCI; ^bp<0.01 vs NCI; ^cp<0.001 vs NCI; ^dp<0.05 vs MCI. Abbreviations: NC=normal cognition, MCI=mild cognitive impairment, VLT=verbal learning task; LDST=letter-digit substitution test; CST=concept shifting test. [§] Neuropsychological data were missing for one case; ^{*}data were missing: LDST, n=4; Stroop 1+2, n=3, CST 1+2, n=20; CST interference, n=22; Stroop interference, n=4.

Table S3a: regional leakage rate for each pooled diagnostic group

	<u>Leakage rate ($K_i(10^{-7} \text{ min}^{-1})$)</u>			
	NC(n=32)	MCI(n=34)	Dementia (n=14)	p-value
Total GM	9.28 [4.49;15.8]	7.08 [4.39;25.8]	7.36 [1.94;12.1]	0.660
Total WM	12.5 [5.37;27.3]	18.6 [3.70;34.0]	8.10 [5.45;18.3]	0.762
Cortex	9.83 [4.07;17.1]	8.47 [4.94;26.4]	7.42 [1.88;11.9]	0.510
DeepGM	3.78 [0.05;23.2]	0.39 [0.09;17.2]	0.75 [0.00;23.5]	0.619
Hippocampus	7.23 [0.18;29.7]	2.89 [0.43;12.5]	4.47 [0.79;40.8]	0.367
NAWM	12.1 [5.32;27.3]	18.7 [3.71;33.8]	7.97 [5.57;18.7]	0.780
WMH	12.6 [0.51;29.6]	13.1 [0.63;45.4]	10.2 [3.23;42.9]	0.847

Values represent median [interquartile range]. No significant group differences were detected. NC=normal cognition, MCI=mild cognitive impairment, GM=gray matter, NAWM=normal appearing white matter, WMH=white matter hyperintensities.

Table S3b: regional leakage volume for each pooled diagnostic group

	<u>Leakage volume ($V_i(\%)$)</u>			
	NC (n=32)	MCI (n=34)	Dementia (n=14)	p-value
Total GM	2.62 [1.35;5.81]	1.87 [1.13;8.71]	2.09 [0.48;4.30]	0.676
Total WM	7.44 [2.67;21.0]	9.74 [1.81;25.3]	5.57 [2.30;15.1]	0.920
Cortex	2.76 [0.99;6.50]	2.34 [1.26;7.68]	2.04 [0.50;3.80]	0.480
DeepGM	1.75 [0.01;12.3]	0.11 [0.02;11.4]	0.25 [0.00;17.2]	0.622
Hippocampus	2.11 [0.04;22.1]	0.79 [0.11;5.31]	2.18 [0.24;24.6]	0.371
NAWM	7.44 [2.61;21.1]	9.75 [1.90;25.1]	5.56 [2.24;15.3]	0.939
WMH	8.14 [0.21;19.7]	7.45 [0.16;28.5]	6.52 [1.24;28.8]	0.954

Values represent median [interquartile range]. No significant group differences were detected. Abbreviations: NC=normal cognition, MCI=mild cognitive impairment, GM=gray matter, NAWM=normal appearing white matter, WMH=white matter hyperintensities.

Table S4: Interaction analysis between diagnostic group and regional BBB leakage in multiple linear regression models with WMH volume as dependent variable

<u>Interaction MCI diagnosis x leakage (n=34)</u>						
	K _i	95% ci	p-value	V _i	95% ci	p-value
Total GM	-0.462	[-1.136;0.212]	0.18	-0.387	[-0.941;0.167]	0.17
Total WM	-0.330	[-0.975;0.315]	0.32	-0.251	[-0.789;0.287]	0.36
Cortex	-0.520	[-1.215;0.174]	0.15	-0.464	[-1.044;0.115]	0.12
DeepGM	-0.014	[-0.414;0.386]	0.95	0.018	[-0.332;0.367]	0.92
Hippocampus	-0.034	[-0.427;0.360]	0.87	-0.077	[-0.416;0.263]	0.66
NAWM	-0.337	[-0.987;0.313]	0.31	-0.262	[-0.804;0.229]	0.35
WMH	-0.185	[-0.687;0.316]	0.47	-0.115	[-0.529;0.299]	0.59
<u>Interaction dementia diagnosis x leakage (n=14)</u>						
	K _i	95% ci	p-value	V _i	95% ci	p-value
Total GM	-0.284	[-0.821;0.252]	0.30	-0.269	[-0.709;0.170]	0.23
Total WM	-0.333	[-0.891;0.226]	0.24	-0.228	[-0.694;0.238]	0.34
Cortex	-0.308	[-0.846;0.230]	0.27	-0.314	[-0.739;0.152]	0.20
DeepGM	-0.140	[-0.495;0.216]	0.44	-0.130	[-0.439;0.178]	0.41
Hippocampus	-0.084	[-0.508;0.341]	0.70	-0.120	[-0.478;0.237]	0.51
NAWM	-0.343	[-0.904;0.217]	0.23	-0.239	[-0.706;0.229]	0.32
WMH	-0.220	[-0.708;0.268]	0.38	-0.138	[-0.536;0.261]	0.50

Values represent standardized beta coefficients with 95% confidence intervals. Reference group is no cognitive impairment group. Covariates in the model included: age, main effect regional BBB leakage, main effect diagnostic group.

Abbreviations: MCI=mild cognitive impairment, K_i=leakage rate, v_i=leakage volume, ci=confidence interval, GM=gray matter, NAWM=normal appearing white matter; WMH=white matter hyperintensities.

Table S5: Interaction effect between diagnostic group and hippocampal BBB leakage in multiple linear regression models with HV as dependent variable

<u>Interaction MCI diagnosis x leakage (n=34)</u>						
	K _i	95% ci	p-value	V _i	95% ci	p-value
Hippocampus	-0.055	[-0.372;0.262]	0.73	0.014	[-0.316;0.344]	0.93
<u>Interaction dementia diagnosis x leakage (n=14)</u>						
	K _i	95% ci	p-value	V _i	95% ci	p-value
Hippocampus	-0.197	[-0.518;0.124]	0.23	-0.178	[-0.525;0.169]	0.32

Values represent standardized beta coefficients with 95% confidence intervals. Reference group is no cognitive impairment group. Covariates in the model included: age, main effect regional BBB leakage, main effect diagnostic group. Abbreviations: MCI=mild cognitive impairment, ci= confidence interval, K_i=leakage rate, V_i=leakage volume.

Table S6a: Mediation statistics on the leakage – memory performance relationships

Total effect		Mediation model effects										
<u>Direct c path</u>		<u>Direct c' path</u>			<u>Mediation a path</u>			<u>Mediation b path</u>				
<u>leakage → memory</u>		<u>leakage → memory</u>			<u>leakage → HV</u>			<u>HV → memory</u>				
B	Wald Z	p-value	B	Wald Z	p-value	B	Wald Z	p-value	B	Wald Z	p-value	
Ki	-18.759	-0.361	0.718	-38.36	-0.711	0.477	6.026	1.689	0.091	3.252	1.720	0.085
[95% CI]	[-124.913;79.030]			[-144.967;65.957]			[-1.147;12.908]			[-0.440;6.933]		
V1	-0.212	-0.170	0.865	-0.609	-0.474	0.635	0.126	1.581	0.114	3.165	1.512	0.131
[95% CI]	[-2.838;2.052]			[-3.256;1.735]			[0.000;0.142]			[-0.037;0.289]		

Mediation analyses were performed with bootstrap standard errors and bootstrap based p-values. Confidence intervals were computed with bias-corrected and accelerated method. The number of bootstrap iterations was set to 5000. All models are corrected for age (at MRI), sex, educational level, time between MRI and neuropsychological assessment, and pooled diagnostic group. Abbreviations: IPS=information processing speed, WMH= white matter hyperintensity volume, B=unstandardized coefficient estimate, CI=confidence interval, Ki=leakage rate, Vi=leakage volume.

Table S6b: Mediation statistics on the leakage – information processing speed performance relationships

Total effect		Mediation model effects										
<u>Direct c path</u> <u>leakage → IPS</u>		<u>Direct c' path</u> <u>leakage → IPS</u>			<u>Mediation a path</u> <u>leakage → WMH</u>			<u>Mediation b path</u> <u>WMH → IPS</u>				
	B	Wald Z	p-value	B	Wald Z	p-value	B	Wald Z	p-value	p-value		
K _i	-577.74	-2.171	0.030	-355.26	-1.325	0.185	35.23	1.980	0.048	-6.31	-3.844	<0.001
[95% CI]	[-1100.563;-40.864]			[-899.347;166.543]			[1.073;71.452]			[-9.332;-2.866]		
V _i	-10.75	-1.789	0.074	-6.11	-1.05	0.295	0.737	1.831	0.067	-6.50	-4.098	<0.001
[95% CI]	[-22.50;1.11]			[-17.70;5.79]			[-0.037;1.439]			[-9.520;-3.295]		

Mediation analyses were performed with bootstrap standard errors and bootstrap based p-values. Confidence intervals were computed with bias-corrected and accelerated method. The number of bootstrap iterations was set to 5000. All models are corrected for age (at MRI), sex, educational level, time between MRI and neuropsychological assessment, and pooled diagnostic group. Abbreviations: IPS=information processing speed, WMH= white matter hyperintensity volume, B=unstandardized coefficient estimate, CI=confidence interval, K_i=leakage rate, V_i=leakage volume.

Table S7: Interaction effect between diagnostic group and BBB leakage in multiple linear regression models with cognitive performance as dependent variable

<u>Interaction MCI diagnosis x leakage (n=34)</u>				
	K_i	95% ci	V_l	95% ci
Hippocampus	-255.6	[-579.4:130.1]	-6.3	[-13.7:2.1]
White matter	-423.4	[-1324:643]	-13.2	[-33.7:9.9]
<u>Interaction dementia diagnosis x leakage (n=10)</u>				
	K_i	95% ci	V_l	95% ci
Hippocampus	-29.8	[-397.96:315.29]	-2.1	[-9.8:5.8]
White matter	-1342.0	[-3471:666]	-35.1	[-76.2:4.33]

Values represent unstandardized regression coefficients obtained with non-parametric bootstrapping, with bias-corrected and accelerated 95% confidence intervals (number of iterations =5000). The reference group is the no cognitive impairment group. The dependent variable is memory performance when hippocampal leakage is the independent variable, and information processing speed when white matter leakage is the independent variable. Covariates in the model included: age (at MRI), main effect regional BBB leakage, main effect diagnostic group, log transformed and normalized white matter hyperintensity volume, educational level, gender and time between neuropsychological assessment and MRI). Abbreviations: MCI=mild cognitive impairment, K_i =leakage rate, ci=confidence interval, v_l =leakage volume.

CHAPTER 4

PERICORTICAL ENHANCEMENT ON DELAYED FLUID- ATTENUATED INVERSION RECOVERY IMAGES IN NORMAL AGING, MILD COGNITIVE IMPAIRMENT, AND ALZHEIMER DISEASE

American Journal of Neuroradiology 2017;38(9):1742-1747.

Whitney M. Freeze, Roald S. Schnerr, Walter M. Palm, Jacobus F. Jansen, Heidi I.L. Jacobs, Erik
I. Hoff, Frans R. Verhey and Walter H. Backes

ABSTRACT

Introduction: Breakdown of BBB integrity occurs in dementia and may lead to neurodegeneration and cognitive decline. We assessed whether extravasation of gadolinium chelate could be visualized on delayed post-contrast FLAIR images in older individuals with and without cognitive impairment.

Methods: 74 individuals participated in this study (15 with Alzheimer disease, 33 with mild cognitive impairment, and 26 with normal cognition). We assessed the appearance of pericortical enhancement after contrast administration, MRI markers of cerebrovascular damage, and medial temporal lobe atrophy. Three participants who were positive for pericortical enhancement (one with normal cognition and two with mild cognitive impairment) were followed up for approximately 2 years. *In vitro* experiments with a range of gadolinium concentrations served to elucidate the mechanisms underlying the post-contrast FLAIR signals.

Results: Post-contrast pericortical enhancement was observed in 21 participants (28%), including 6 individuals with Alzheimer disease (40%), 10 with mild cognitive impairment (30%), and 5 with normal cognition (19%). Pericortical enhancement was positively associated with age ($p < 0.02$) and ischemic stroke ($p < 0.05$), but not with cognitive status ($p = 0.3$). Foci with enhanced signal remained stable over time in all follow-up cases. The *in vitro* measurements confirmed that FLAIR imaging is highly sensitive for the detection of low gadolinium concentrations in CSF, but not in cerebral tissue.

Conclusion: Post-contrast pericortical enhancement on FLAIR images occurs in older individuals with normal cognition, mild cognitive impairment and dementia. It may represent chronic focal superficial BBB leakage. Future longitudinal studies are needed to determine its clinical significance.

BACKGROUND

Alzheimer disease (AD) is the most common form of dementia, and clinically characterized by progressive cognitive decline and associated functional impairment in daily living. Accumulating evidence suggests that microvascular dysregulation and BBB disruption are early events in the multifactorial disease cascade that leads to continuous neuronal loss ^{1,2}. Over the past decades, the development of gadolinium-based contrast agents has enabled detailed *in vivo* examination of BBB leakage by detection of extravasated gadolinium with post-contrast T1-weighted imaging (T1WI). In this light, previous studies report subtle changes in vascular permeability in the cortex, white matter, and hippocampus in early-stage AD ^{3,4}. Small changes in the dynamics of contrast agent leakage in ventricular cerebrospinal fluid (CSF) have also been reported in AD ⁵. It is likely that vascular permeability changes also occur in vessels adjacent to CSF along the cortical surface or leptomeninges. However, T1-weighted BBB permeability imaging around the cortex in disease conditions with subtle changes to BBB integrity is probably difficult because the leptomeninges are highly vascularized and become relatively strongly enhanced due to high gadolinium concentrations within the blood vessels. Furthermore, when gadolinium chelate leaks into CSF, convection and diffusion may dilute the contrast agent at concentrations that become too low to detect.

Delayed post-contrast fluid-attenuated inversion recovery (FLAIR) imaging yields up to 10-fold higher sensitivity compared to post-contrast T1WI for the detection of low amounts of gadolinium chelate in CSF ^{6,7}. Previous studies that applied post-contrast FLAIR imaging report pericortical gadolinium leakage that appears as signal enhancement in the CSF in various clinical disease conditions with BBB leakage, including acute ischemic stroke and MS ^{8,9}. The application of the post-contrast FLAIR technique has not yet been evaluated in memory clinic patients and in normal aging.

The primary objective of this study was to explore the utility of post-contrast FLAIR imaging in identifying pericortical enhancement in AD, mild cognitive impairment (MCI), and age-matched cognitively normal individuals. We hypothesized that pericortical BBB leakage is more common in AD and MCI compared with cognitively normal individuals, and that it is associated with MRI markers of cerebrovascular damage. Finally, to investigate whether signal alterations could result from leakage of contrast medium into cortical tissue or into the CSF, we performed *in vitro* experiments to mimic the FLAIR signal effects of different concentrations of gadolinium in CSF and cerebral tissue.

METHODS

Participants

Patients were prospectively recruited at two memory clinics (Maastricht University Medical Center and Zuyderland Medical Center Heerlen) between November 2014 and December 2016, and healthy controls were enrolled via a local newspaper advertisement in the same period. Detailed neuropsychological assessment, medical history, and educational level ¹⁰ were recorded for all participants. Inclusion criteria for patients were clinical diagnosis of subjective cognitive decline ¹¹, MCI ¹² or AD ¹³, and mini-mental state examination (MMSE) ¹⁴ score ≥ 20 . Control participants reported no subjective cognitive impairment and were screened to ensure cognitive health (MMSE score ≥ 27). Inclusion criteria for all participants were age ≥ 55 years and eligibility for undergoing MR imaging and gadolinium contrast administration. Exclusion criteria for all participants included impaired renal function (estimated glomerular filtration rate < 30 mL/min), and presence of psychiatric or neurological disorders that might have caused the cognitive impairment, other than AD, cerebral small vessel disease, or non-invalidating stroke. Participants with recent (e.g. < 3 months before inclusion) ischemic or hemorrhagic stroke were excluded. Informed consent was obtained from all participants prior to participation. This study was carried out in accordance with the rules and regulations of the local institutional review boards and approved by the medical ethics committee in Maastricht.

MR imaging protocol

MR images were acquired on a 3.0T MRI system with a 32-channel head coil (Philips, Achieva TX; Philips Healthcare, Best, the Netherlands). The imaging protocol was relatively long because other sequences that are not relevant to this paper were included, and was split into two separate sessions for the participants comfort. During the first session without contrast administration, three-dimensional T1-weighted gradient echo (TR/TE of 8/4ms; acquisition matrix of 256x256; 160 slices; 1mm slice thickness) and three-dimensional T2-FLAIR (TR/TE/TI of 4800/290/1650ms; acquisition matrix of 256x256; 200 slices; 1mm slice thickness) images were acquired, as well as multi-slice T2-weighted TSE and T2-weighted fast field echo images with a slice thickness of 5 mm in the transverse plane. During a second session (median time between scans was 2 days, interquartile range 0-5.5) the same FLAIR sequence was acquired 16 minutes after intravenous administration of 0.1 mmol/kg gadobutrol in the antecubital vein (injection rate 3 mL/s, 20 mL saline flush).

Image analysis

Pericortical enhancement was defined as hyperintense signal substantially brighter than the proximal parenchymal signal intensity, occurring in the subarachnoid space on post-contrast FLAIR images, but not on pre-contrast images. Two raters blinded for clinical data (W.M.F., W.M.P.) independently evaluated the scans for the presence of pericortical enhancement. Discrepancies and cases of uncertainty (Cohen's $\kappa=0.50$) were evaluated during consensus meetings, after which both raters agreed on all cases. When present, pericortical enhancement was classified according to vascular territory (anterior, middle or posterior cerebral artery), hemisphere (left/right), location (within a sulcus, along the cortical convexity, or within the longitudinal fissure) and number of foci.

White matter hyperintensities were scored on pre-contrast FLAIR images according to the Fazekas scale ¹⁵. Old lacunar infarcts were scored as subcortical round or ovoid structures of 3-15mm in diameter with low signal intensity on FLAIR images, and CSF-like signal intensity on T2WI ¹⁶. Cerebral microbleeds were scored as round or ovoid areas of signal void with associated blooming on T2-weighted fast field echo images in cortical or subcortical regions, excluding potential mimics such as bilateral iron/calcium deposits in the basal ganglia, bone, or vessel flow voids ¹⁷. Old ischemic stroke of >15mm was scored on T2-weighted TSE and FLAIR images, and old cerebral hemorrhage was scored on T2-weighted fast field echo images. Medial temporal lobe atrophy (MTA) was rated bilaterally on coronal T1WI using a previously described visual rating scale ¹⁸. The average score of the left and right side was taken as final MTA score. Vascular markers and MTA were scored by an experienced neuroradiologist (W.M.P.), who was blinded to clinical data.

Genetic risk factor assessment

Blood samples were collected and apolipoprotein E (ApoE) genotyping was performed using a method of polymerase chain reaction. Individuals with at least one $\epsilon 4$ allele were classified as ApoE4 positive.

Follow-up

One cognitively normal participant and two patients with MCI who were positive for pericortical enhancement were followed-up after 21 to 22 months. The same scan protocol was applied as described earlier but all scans were acquired in one single session.

Statistical analyses

Differences between individuals with and without pericortical enhancement were assessed with chi-square or Fisher's exact tests (when applicable) for categorical variables. For continuous variables with a normal data distribution, independent two-sample t-tests were performed to compare the means of the two groups. Mann-Whitney U tests were performed in case of non-normal data distribution.

To assess differences between the diagnostic groups (cognitively normal, MCI, and AD), ANOVA was performed for continuous variables with normal data distribution, and Kruskal-Wallis tests were performed for continuous variables with non-normal data distribution. Chi-square or Fisher's exact test were used for categorical variables. A threshold of $\alpha < 0.05$ was used to determine statistical significance, all p-values are two-tailed and analyses were conducted using SPSS statistical software (version 24.0; IBM Corp., Armonk, NY).

In vitro experiment

Gadobutrol was dissolved in demineralized water in plastic falcon tubes (50mL) in different concentrations (0.004, 0.008, 0.016, 0.031, 0.063, 0.125, 0.25, 0.5, 1.0 mmol/L), mimicking *in vivo* gadolinium concentrations with a range of leakage severity in CSF. The same gadolinium concentrations were adopted for a solution mimicking cerebral tissue, which was prepared by dissolving 0.08 mM MnCl_2 in demineralized water. The T1 and T2 relaxation times of this MnCl_2 solution were determined using signal intensities from inversion recovery TSE images with TR=3000ms and varying TI [50, 100, 200, 400, 600, 800, 1000, 1500ms], and T2W images with fixed TR=453ms and varying TE [20, 40, 60, 80, 100, 120, 140, 160, 180, 200ms]. The T1 and T2 relaxation times (figure S1) of the tissue phantom approximated previously reported values of cerebral cortex at 3 Tesla with 1170 ms and 122 ms, respectively ¹⁹.

The samples mimicking CSF and tissue with different concentrations of gadolinium were scanned with the T1 and FLAIR sequences described above.

RESULTS*Neuroradiological imaging*

74 participants with a mean age of 71.2 years (SD $\pm$ 6.2) were enrolled in this study, including 26 cognitively normal participants, (13 healthy controls and 13 with subjective cognitive decline), 33 with MCI, and 15 with AD. Participant characteristics according to cognitive status (normal cognition, MCI or AD) are provided in supplementary table S1.

Table 1: Characteristics of pericortical enhancement

	Pericortical enhancement		
	Yes (n=21)	No (n=53)	p-value
Demographics			
Age, mean(SD),y	73.5(4.8)	70.2(6.4)	0.02
Female sex, No(%)	10(48)	22(42)	0.8
Clinical subtype, No(%)			0.3
Normal cognition	5(19)	21(81)	
MCI	10(30)	23(70)	
AD	6(40)	9(60)	
MRI characteristics			
Fazekas score, median(IQR)	1.0(1.0-3.0)	1.0(1.0-2.0)	0.4
Lacunar infarct present, No(%)	7(33)	8(15)	0.11
Ischemic stroke present, No(%)	4(19)	3(6)	0.18
Lacunar and/or ischemic stroke present, No(%)	9(43)	10(19)	0.04
Microbleed present, No(%)	2(10)	7(13)	1.0
Hemorrhagic stroke present, No(%)	1(5)	1(2)	1.0
MTA score, median(IQR)	1.0(0.5-2.25)	0.5(0-1.5)	0.08
Gadobutrol dosage, mean(SD), mL	7.3(1.2)	7.3(1.1)	0.98
Cognitive performance			
MMSE, median(IQR)§	28(25-28)	28(26-29)	0.97
Genotype			
ApoE4 positive*, No(%)	6(30)	25(47)	0.3

IQR=interquartile range; SD=standard deviation; *Data were missing for one case; §Data were missing for two cases

Twenty-one individuals showed evidence of abnormal pericortical enhancement on post-contrast FLAIR images (figure 1). Overall there were thirty-two foci, among which ten (31%) were located within the posterior, nine (28%) within the middle, and thirteen (41%) within the anterior cerebral artery territory. Fifteen foci (47%) were located in the left, and seventeen (53%) in the right hemisphere. Most foci were located on the cortical convexity (12/32, 38%) or within a sulcus (12/32, 38%), but one (3%) traversed several sulci and seven (22%) were located within the longitudinal fissure. Pericortical enhancement was observed as a single focus in most cases (13/21,

62%). Three cases with MCI and two cases with dementia showed two foci, and one case with MCI and two cases with AD showed three foci (figure 2).

In the three enhancement-positive cases with a follow up time between 21 and 22 months, the foci remained stable over time regarding location, size and shape (figure 1).

Figure 1. Pericortical enhancement is evident on sagittal, coronal, and transverse post-contrast FLAIR images of a 76-year-old male patient with MCI. No signal enhancement is apparent on the pre-contrast FLAIR image (upper- right) (A). Pericortical enhancement remains stable over time in a male healthy control participant aged 87 years at baseline (B) and in a male MCI participant aged 71 at baseline (C).

In addition to the pericortical foci, one focus of signal enhancement was found in what appeared as a perivascular space on the post-contrast, but not on the pre-contrast FLAIR images (figure S2). No signal attenuation was observed in other fluid-containing structures including lacunar infarcts, the ventricular system, or the eyes.

The group characteristics of individuals with pericortical enhancement are summarized in table 1. The prevalence per diagnostic group was 5/26 (19%) in cognitively normal individuals, 10/33 (30%) in individuals with MCI, and 6/15 (40%) in individuals with AD. Although the prevalence of pericortical enhancement was higher in the AD and MCI group compared with the cognitively normal individuals there were no statistically significant group differences (Fisher's exact test, $p=0.3$). Individuals with pericortical enhancement were significantly older ($t(50)=2.4$, $p<0.02$), and more often had ischemic stroke ($X^2=4.5$, $df=2$, $p<0.05$) when lacunar infarct and ischemic stroke data were pooled. No associations were apparent between pericortical enhancement and sex, amount of contrast agent administered, MMSE score, ApoE genotype, and

other MRI markers of cerebrovascular damage (see Table 1). The location of pericortical enhancement did not correspond to the location of other MRI markers of cerebrovascular damage.

Figure 2. Two foci of pericortical enhancement on transverse (A) and coronal (B) post-contrast FLAIR images in a 73-year-old female participant with AD.

In vitro measurements

The phantom imaging experiment shows that relatively low concentrations of gadobutrol in CSF appear bright on FLAIR images, while at higher concentrations the signal declines. On T1W1 low concentrations of gadolinium in CSF appear dark while at higher concentrations the signal increases. In contrast, the FLAIR images of tissue phantoms with low gadolinium concentrations show only very little signal enhancement while the signal decreases at higher concentrations. On T1WI a gradual signal increase can be observed with increasing gadobutrol concentrations (figure S3 and figure S4).

DISCUSSION

Delayed post-contrast FLAIR imaging revealed pericortical signal enhancement in 28% of all participants who underwent MR imaging. This signal enhancement likely results from leakage of gadolinium chelate through cortical or leptomeningal vessels and as such represents a marker for

focal loss of BBB integrity. Although pericortical enhancement occurred more often in MCI or AD compared with cognitively normal individuals, there were no statistical group differences. Pericortical enhancement was associated with increasing age and ischemic stroke. Interestingly, in the three cases that were followed up, the pericortical enhancement remained stable over a period of 21-22 months. A possible explanation for this imaging phenomenon is chronic inflammation with BBB disruption in the leptomeninges or superficial cortical vessels.

The potential of post-contrast FLAIR imaging for the detection of contrast agent leakage through the BBB was already described by Mathews and colleagues in 1999²⁰. Since then, several studies on the application of this technique have been published. Many of these describe post-ischemic diffuse enhancement in CSF in one or both hemispheres, a phenomenon called hyperintense acute reperfusion marker^{7,9,21,22}. Similar to its acute presentation after ischemic stroke or transient ischemic attack, appearance of hyperintense acute reperfusion marker has also been described after hemorrhagic stroke, cardiac surgery, and carotid revascularization²³⁻²⁵. These findings suggest a common process of rapid pericortical BBB leakage following cerebral ischemia or hypoxia. In addition to its relationship with ischemia, pericortical BBB leakage on post-contrast FLAIR images has been described in infectious diseases of the CNS, including meningitis and MS^{8,26}. Together, these studies suggest that (chronic) hypoxic and/or inflammatory processes in the cortex and leptomeninges may underlie this neuroimaging phenomenon. Pericortical enhancement is likely not specific for a particular disease and also occurs in the context of normal aging^{27,28}.

We found no association between pericortical enhancement and diagnostic group, ApoE genotype, MTA score or global cognition. Hence, the clinical relevance of pericortical enhancement in our study population remains unclear. Although delayed post-contrast FLAIR imaging is not routinely applied in clinical examination, it is important to note that the CSF signal change should not be confused with other causes of signal increase in the subarachnoid space on FLAIR images such as subarachnoid hemorrhage or meningeal metastasis²⁹.

Because the signal enhancement on post-contrast FLAIR images appears very close to the cortical surface we aimed to assess whether the focal enhancement occurs in CSF or in cortical tissue. Our phantom study shows that FLAIR imaging is relatively insensitive for detection of gadolinium in brain tissue, while strong signal enhancement is observed in CSF mimicking phantom solutions at low gadolinium concentrations (i.e. 10-500 $\mu\text{mol/L}$). We therefore interpret the pericortical enhancement as leakage and accumulation of low gadolinium concentrations in the pericortical CSF.

Previous studies have shown two advantages of post-contrast FLAIR imaging over T1WI in detecting pericortical BBB leakage. First, post-contrast FLAIR imaging is superior to post-

contrast T1WI in detecting low gadolinium concentrations (i.e. 10-500 $\mu\text{mol/L}$) in CSF^{7, 20}. Because of the long T1 relaxation time of CSF, FLAIR is relatively sensitive to subtle shortening of the T1 relaxation time caused by low concentrations of gadolinium. Secondly, previous work has shown that, unlike T1WI, FLAIR is rather insensitive to the effects of contrast medium within blood vessels as the shortening of the T2 relaxation time at higher gadolinium concentrations and the effects of blood flow both result in a strong signal decay²⁶.

Strengths of this study include the availability of both pre- and post-contrast FLAIR images obtained in individuals with a wide range of cognitive performance. The availability of other MRI sequences allowed us to look for associations between post-contrast FLAIR enhancement and various markers of cerebrovascular damage. The time frame of 16 minutes between contrast injection and FLAIR imaging proved suitable to detect pericortical enhancement in a relatively high number (i.e. 28%) of participants in our study. Limitations of this study include the relatively small sample size, which limits our ability to draw conclusions about the presence or absence of associations between BBB leakage and variables of interest. Because we did not include markers that are more specific for neurodegeneration than MTA and AD biomarkers amyloid-beta and tau were not measured, their association with pericortical enhancement remains unknown. Although the sensitivity of post-contrast FLAIR imaging compared with T1WI was assessed in our phantom study, we did not set out to make this comparison *in vivo*. We expect that the enhancement of the leptomeningeal vessels due to intravascular gadolinium complicates the detection of focal pericortical enhancement on post-contrast T1-weighted images.

Future studies are needed to elucidate the pathophysiology and clinical relevance of pericortical BBB leakage in relation to neurodegeneration. The potential of pericortical enhancement as a biomarker for cognitive decline and dementia could be explored by future studies that compare the cognitive profile of individuals with and without pericortical enhancement within the same diagnostic group (i.e. normal cognition, MCI, dementia) and by following individuals over time to assess conversion rate to MCI or dementia. The optimal time frame between contrast injection and scan acquisition remains to be explored, as well as whether this phenomenon also occurs in younger individuals. In prospect, larger study samples and longitudinal studies may shed light on the clinical consequences following signs of pericortical BBB disruption in aging, dementia, and other disorders of the CNS.

CONCLUSION

This study identified focal pericortical BBB leakage on delayed post-contrast FLAIR images in a substantial proportion of AD and MCI patients as well as cognitively normal participants.

Pericortical signal enhancement was associated with increasing age and previous ischemic stroke. In all three follow-up cases the focal BBB leakage persisted and remained stable over time. The results of our study demonstrate the suitability of post-contrast FLAIR imaging as a tool for detecting possibly chronic pericortical vascular leaks in a memory clinic setting and in cognitively normal older individuals.

REFERENCES

1. Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nature reviews Neuroscience* 2011;12:723-738
2. Iturria-Medina Y, Sotero RC, Toussaint PJ, et al. Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. *Nat Commun* 2016;7:11934
3. Montagne A, Barnes SR, Sweeney MD, et al. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* 2015;85:296-302
4. van de Haar HJ, Burgmans S, Jansen JF, et al. Blood-Brain Barrier Leakage in Patients with Early Alzheimer Disease. *Radiology* 2016;152244
5. Starr JM, Farrall AJ, Armitage P, et al. Blood-brain barrier permeability in Alzheimer's disease: a case-control MRI study. *Psychiatry research* 2009;171:232-241
6. Mamourian AC, Hoopes PJ, Lewis LD. Visualization of intravenously administered contrast material in the CSF on fluid-attenuated inversion-recovery MR images: an in vitro and animal-model investigation. *AJNR American journal of neuroradiology* 2000;21:105-111
7. Kohrmann M, Struffert T, Frenzel T, et al. The hyperintense acute reperfusion marker on fluid-attenuated inversion recovery magnetic resonance imaging is caused by gadolinium in the cerebrospinal fluid. *Stroke* 2012;43:259-261
8. Absinta M, Vuolo L, Rao A, et al. Gadolinium-based MRI characterization of leptomeningeal inflammation in multiple sclerosis. *Neurology* 2015;85:18-28
9. Latour LL, Kang DW, Ezzeddine MA, et al. Early blood-brain barrier disruption in human focal brain ischemia. *Annals of neurology* 2004;56:468-477
10. Verhage F. Intelligence and Age in a Dutch Sample. *Human Development* 1965;8:238-245
11. Jessen F, Amariglio RE, van Boxtel M, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2014;10:844-852
12. Petersen RC. Mild cognitive impairment as a diagnostic entity. *Journal of internal medicine* 2004;256:183-194
13. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34:939-944
14. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research* 1975;12:189-198
15. Fazekas F, Chawluk JB, Alavi A, et al. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol* 1987;149:351-356
16. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *The Lancet Neurology* 2013;12:822-838
17. Greenberg SM, Vernooij MW, Cordonnier C, et al. Cerebral microbleeds: a guide to detection and interpretation. *The Lancet Neurology* 2009;8:165-174
18. Scheltens P, Leys D, Barkhof F, et al. Atrophy of medial temporal lobes on MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. *Journal of neurology, neurosurgery, and psychiatry* 1992;55:967-972
19. Wansapura JP, Holland SK, Dunn RS, et al. NMR relaxation times in the human brain at 3.0 tesla. *Journal of magnetic resonance imaging : JMRI* 1999;9:531-538
20. Mathews VP, Caldemeyer KS, Lowe MJ, et al. Brain: gadolinium-enhanced fast fluid-attenuated inversion-recovery MR imaging. *Radiology* 1999;211:257-263
21. Warach S, Latour LL. Evidence of reperfusion injury, exacerbated by thrombolytic therapy, in human focal brain ischemia using a novel imaging marker of early blood-brain barrier disruption. *Stroke* 2004;35:2659-2661
22. Forster A, Wenz H, Groden C. Hyperintense Acute Reperfusion Marker on FLAIR in a Patient with Transient Ischemic Attack. *Case reports in radiology* 2016;2016:9829823
23. Kidwell CS, Burgess R, Menon R, et al. Hyperacute injury marker (HARM) in primary hemorrhage: A distinct form of CNS barrier disruption. *Neurology* 2011;77:1725-1728
24. Merino JG, Latour LL, Tso A, et al. Blood-brain barrier disruption after cardiac surgery. *American Journal of Neuroradiology* 2013;34:518-523

25. Cho AH, Suh DC, Kim GE, et al. MRI evidence of reperfusion injury associated with neurological deficits after carotid revascularization procedures. *European journal of neurology : the official journal of the European Federation of Neurological Societies* 2009;16:1066-1069
26. Fukuoka H, Hirai T, Okuda T, et al. Comparison of the added value of contrast-enhanced 3D fluid-attenuated inversion recovery and magnetization-prepared rapid acquisition of gradient echo sequences in relation to conventional postcontrast T1-weighted images for the evaluation of leptomeningeal diseases at 3T. *AJNR American journal of neuroradiology* 2010;31:868-873
27. Erdo F, Denes L, de Lange E. Age-associated physiological and pathological changes at the blood-brain barrier: A review. *J Cereb Blood Flow Metab* 2017;37:4-24
28. Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease--systematic review and meta-analysis. *Neurobiology of aging* 2009;30:337-352
29. Stuckey SL, Goh TD, Heffernan T, et al. Hyperintensity in the subarachnoid space on FLAIR MRI. *AJR Am J Roentgenol* 2007;189:913-921

SUPPLEMENTARY MATERIAL

Table S1: Participant characteristics

	Normal cognition (n=26)	MCI (n=33)	AD (n=15)
Demographics			
Age, mean(SD), y	73.31(5.5)	69.21(6.2) ^a	71.67(6.3)
Female sex, No(%)	12(46)	16(48)	4(27)
Education, mean(SD)	5.27(1.3)	4.76(1.1)	4.80(1.5)
ApoE4 positive, No(%) [*]	10(38)	14(44)	7(47)
MRI characteristics			
Fazekas, median(IQR)	1.0(1.0-2.0)	2.0(1.0-2.0)	1.0(1.0-2.0)
Microbleeds present, No(%)	0(0)	6(18) ^a	3(20) ^a
Lacunes present, No(%)	5(19)	7(21)	3(20)
Ischaemic stroke present, No(%)	1(4)	6(18)	0(0)
Lacunar and/or ischaemic stroke present, No(%)	5(19)	11(33)	3(20)
Haemorrhagic stroke present, No(%)	0(0)	1(3)	1(7)
MTA score, median(IQR)	0.50(0-1)	1.0(0-1.5)	1.50(0.5-2.5) ^a
Pericortical enhancement, No(%)	5(19)	10(30)	6(40)
Cognition			
MMSE, median(IQR) [*]	29(28-30)	27(26-29.5) ^a	24(21-27) ^{ab}

Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; MTA, medial temporal lobe atrophy; MMSE, mini-mental state examination; SD, standard deviation; IQR, interquartile range

^ap<0.05 vs normal cognition; ^bp<0.05 vs MCI;

^{*}Data were missing: ApoE4 positive n=73; MMSE n=72

Figure S1. Graphs show the signal intensity of a phantom containing 0.08-mmol/L MnCl_2 versus TI and TE on T1- and T2-weighted MR imaging, respectively, and a fit to the data. On inversion recovery turbo spin-echo images with a fixed TR of 3000 ms, the signal intensity increases when the TI increases (*left graph*). On T2-weighted images with a fixed TR of 453 ms, the signal intensity decreases when the TE increases (*right graph*). The calculated T1 and T2 relaxation times of the phantom are 1170 and 122 ms, respectively.

Figure S2. On the precontrast FLAIR image (A), no signal enhancement is apparent in this 72-year old female participant with MCI, while postcontrast enhancement is visible in what appears to be an enlarged perivascular space on the postcontrast FLAIR image (B).

Figure S3. FLAIR and T1-weighted signal intensities of different gadolinium concentrations in phantoms mimicking CSF and cerebral tissue. The FLAIR sequence is more sensitive to the lowest concentrations of gadolinium contrast agent compared with T1WI in phantoms mimicking CSF. At higher concentrations, the FLAIR sequence shows signal decay due to the decreasing T2 relaxation time, while the T1-weighted signal still increases. In phantoms mimicking cerebral tissue, both FLAIR and T1WI are not very sensitive for detecting low concentrations of gadolinium. The signal intensity keeps increasing for higher concentrations of gadolinium contrast agent on T1WI, while there is a signal decay on FLAIR images.

Figure S4: The signal intensity of each phantom containing gadolinium (S1) is divided by the signal intensity of the phantom containing no gadolinium (S0) to show the relative signal intensities for the T1 and FLAIR sequences for phantoms mimicking tissue and CSF. At low gadolinium concentrations (ie, 100–250 μmol), the relative signal intensity on FLAIR images is highest in the CSF phantoms.

CHAPTER 5

BLOOD-BRAIN BARRIER DYSFUNCTION IN SPONTANEOUS INTRACEREBRAL HEMORRHAGE — A SYSTEMATIC REVIEW.

Submitted.

Whitney M. Freeze Heidi I.L. Jacobs, Floris H.B.M. Schreuder, Robert J. van Oostenbrugge,
Walter H. Backes, Frans R. Verhey, and Catharina J.M. Klijn

ABSTRACT

Introduction: This work constitutes a systematic review on blood-brain barrier (BBB) dysfunction in the etiology of spontaneous intracerebral hemorrhage (ICH) due to cerebral small vessel disease (cSVD).

Methods: We searched Medline (1946-2017) and Embase (1974-2017) for animal and human studies reporting on BBB dysfunction associated with ICH or cerebral microbleeds (CMBs).

Results: Of twenty-five eligible studies, ten were animal studies and fifteen were in humans. The authors found indications for BBB dysfunction in all four animal studies addressing HIV-related ICH (n=32), and in four of six studies on CAA-related ICH (n=47). Of the studies in humans, five of six studies in patients with CAA-related ICH (n=117) and seven out of eight studies examining ICH with mixed or unspecified underlying etiology (n=435) found indications for BBB dysfunction. One *post mortem* study in HIV-related ICH (n=82) found no evidence for BBB abnormalities.

Conclusion: Signs of BBB dysfunction were found in twenty out of twenty-five studies. BBB integrity deserves further investigation with a view to identification of potential treatment targets for spontaneous ICH.

BACKGROUND

Spontaneous intracerebral hemorrhage (ICH) is the most disabling and deadliest form of stroke ¹, with an overall case fatality of 40% at 1 month, and 55% at 1 year ^{2,3}. Outcome after ICH has seen little improvement over the past decades ^{2,4}. An important factor that has hampered development of specific treatment and prevention strategies is the lack of knowledge on pathophysiological processes underlying spontaneous ICH. Hypertensive vasculopathy (HV) and cerebral amyloid angiopathy (CAA), two distinct types of cerebral small vessel disease (cSVD), are thought to be the most important causes of spontaneous ICH. In HV, plasma protein leakage into the vessel wall, vascular fibrosis, and the accumulation of lipid-containing macrophages affect the deep penetrating small vessels in the brain ⁵. This type of cSVD is usually associated with ICH located in deep brain structures, including the basal ganglia and thalamus, and in the posterior fossa and brainstem ⁶. CAA is characterized by progressive deposition of amyloid- β (A β) protein in the walls of small to medium sized arteries (including arterioles) of the cerebral cortex and leptomeninges ⁷, and is an important cause of lobar ICH ^{6,8}.

A candidate key player in the pathophysiology of ICH is the blood-brain barrier (BBB) ^{9,10}. The BBB is a term used to describe the unique selective transport regulation properties of the cerebral microvasculature ¹¹. The BBB is located along the microvascular endothelium and consists of endothelial cells connected by tight junctions, which restrict paracellular transport. Neighboring cell types include astrocytes that ensheath the microvascular wall with their endfeet and pericytes that are embedded within the connective endothelial basement membrane. These cells, as part of the neurovascular unit network that regulates local cerebral blood flow, support and interact with the endothelial cells and are essential for the induction and maintenance of the BBB ^{12,13}. Functions of the BBB include the control of molecular traffic to exclude toxins, maintenance of ion homeostasis, and control of immune surveillance and response with minimal inflammation to protect the neuronal environment ¹⁴. Previous research has led to the hypothesis that BBB dysfunction may trigger HV-type cSVD ¹⁵, and accumulating evidence also suggests a pivotal role of BBB dysfunction in CAA-related vessel pathology ¹⁶.

The objective of this systematic review is to summarize and evaluate the currently available evidence from human and animal studies to substantiate a potential role of BBB dysfunction in the underlying vasculopathy of ICH. Cerebral microbleeds (CMBs), as radiological imaging markers of cSVD and independent predictors of future ICH ^{17,18}, may bear a pathophysiological mechanism comparable to ICH ¹⁹. Therefore, we also included studies that investigated BBB integrity in relation to CMBs and their histological equivalent, cerebral microhemorrhages (CMHs) ²⁰.

METHODS

Literature search strategy

Studies were identified via a systematic search of MEDLINE and EMBASE (MEDLINE 1946-11/3/17, EMBASE 1974-11/3/2017). The terms ‘intracerebral hemorrhage’, ‘cerebral microbleed’, or ‘cerebral microhemorrhage’ were entered in combination with the term ‘blood-brain barrier’ (appendix e-1). Conference reports, case reports, reviews, and articles in languages other than English were not included.

Inclusion/exclusion criteria

We included both human and animal studies investigating 1) spontaneous ICH or CMBs/CMHs, and 2) BBB dysfunction preceding the hematoma in time or occurring spatially distant to the hematoma. Exclusion criteria were artificially induced hemorrhage (e.g. in experimental animal models by injection of hemorrhage mimicking or inducing compounds), traumatic ICH, hemorrhagic transformation of infarction, and secondary ICH (due to rupture of an arteriovenous malformation, dural arteriovenous fistula, cavernoma, tumor and others). We also excluded reports that investigated perihematoma BBB leakage or plasma markers of BBB dysfunction in the acute phase after ICH.

Selection of studies and data extraction

One author (W.M.F.) performed title and abstract screening to identify studies that were eligible for inclusion, and subsequently reviewed full-text versions of these studies (figure 1). In case of uncertainty, studies were discussed with a second author (C.J.M.K.). The reference lists of the included articles were screened, but did not lead to the inclusion of additional papers. Data was extracted from all included studies by one author (W.M.F.) and independently by two other authors (F.H.B.M.S. and H.I.L.J.), who each extracted information from half of the included studies, randomly distributed. Discrepancies were resolved in consensus with the three authors (W.M.F., F.H.S., H.I.L.J.). We extracted the following data: (1) study type: animal/human, *in vivo*/*post mortem*, cross-sectional/longitudinal and retrospective/prospective design; (2) hemorrhage type: ICH/CMB/CMH and underlying etiology: HV/CAA; (3) main research methods: imaging vs biochemical and techniques used to assess BBB dysfunction and hemorrhage occurrence; (4) markers used to define BBB dysfunction and hemorrhage, if possible including hemorrhage location; (5) studied sample (percentage with hemorrhage) and control group (size, age, and sex); (6) main outcomes (numeric where possible). To quantify the strength of group differences (when possible), the standardized mean difference (Hedges’ G) was computed for human observational

studies that quantified and reported a mean and SD of the BBB disruption marker for the studied sample and control group. We followed the PRISMA guideline (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

Figure 1: Flow chart of study selection

After title and abstract examination of 2638 publications, 2,400 papers were excluded, leaving 238 papers to be read. Of these, 212 papers did not meet the inclusion criteria (hemorrhage was induced artificially in animals (n=125) or caused by factors other than cSVD (n=15), BBB dysfunction was only assessed in the peri-hematoma region (n=33), or a direct measure for BBB leakage was absent (n=20)), and 4 papers were excluded because we could not translate them (1 Polish and 3 Chinese). One duplicate dataset was discovered, so we only included the first publication. The remaining 25 papers were deemed eligible for inclusion. No additional papers were included after screening the reference lists of the included articles.

Study quality assessment

We assessed the methodological quality of the individual human case-control studies according to the Newcastle-Ottawa Scale (NOS)²¹, and the quality of the studies with a case-series design with an adapted version of the NOS (table e-1)²². Methodological quality of the individual animal studies was rated using an adapted version of the CAMARADES scale (table e-2)²³.

RESULTS

Our search yielded a total of 2638 publications, of which 25 studies were relevant. Ten studies described a total of 79 animals in which cSVD-related hemorrhage was modeled, and 54 animals serving as controls. Fifteen studies described 634 human patients with ICH and CMBs, and 1080 controls without ICH and CMBs. The methodological quality of all studies was assessed and varied from poor (2/9) to good (9/9) (table e-1 and e-2).

Animal studies

Of the ten studies in animals, four assessed BBB dysfunction in HV-related ICH (n=32), and six assessed CAA-related ICH (n=47) (table 1 and table e-3) ²⁴⁻³³.

All four studies of HV-related hemorrhage found evidence for BBB dysfunction ²⁴⁻²⁷. One of these demonstrated increased vascular permeability to a gadolinium-based contrast agent on MRI that preceded ICH in time in 3 out of 7 (42%) stroke-prone spontaneously hypertensive rats (SP-SHR) that developed ICH ²⁴. The location of BBB leakage corresponded to the site of the ICH. The second study assessed proteomic changes in microvessels of transgenic (hCETP) hyperlipidemic Dahl salt sensitive hypertensive rats and their non-transgenic normolipidemic littermates ²⁵. Based on age, sex and transgenic status the rats were divided into being stroke-prone (n=11) and non-stroke prone (n=15). Stroke susceptibility in the pre-stroke stage was associated with an increase in twelve proteins, of which nine were related to BBB integrity. A third study demonstrated CMHs and bilateral increased extravasation of the plasma protein immunoglobulin G (IgG) with co-localized matrix metalloproteinase-9 (MMP-9) immunoreactivity in the external capsule, corpus callosum, and internal capsule in SP-SHR with unilateral carotid artery occlusion and on a stroke-permissive diet (Japanese-style) (n=5) compared with sham-operated SP-SHR on a regular diet (n=5) ²⁷. The fourth study identified deposition of IgG in vessel walls within the cortex, basal ganglia, hippocampus and corpus callosum of SP-SHR (n=9) that preceded the formation of CMHs ²⁶.

Four of six studies examining animal models of CAA found evidence for BBB dysfunction ²⁸⁻³³. One study assessed fibrinoid extravasation in the forebrain cortex of old squirrel monkeys with CAA (n=4) and in a young squirrel monkey with no CAA (n=1) ²⁸. Although very rare, CMHs and fibrinoid extravasation were found in some CAA affected vessels in the old animals while such changes were absent in the young animal. These observations were not quantified. A second study demonstrated BBB leakage in the form of increased albumin and IgG plasma protein extravasation in TG2576 (APP/Sw) (n=10) and bigenic PSAPP (APP/Sw X PS1_{M146L}) (n=6) mice with increased CMH load (from young to old: 0.6-1.67 for albumin and 0.54-1.73 for IgG) compared with non-

transgenic controls (n=6) (from young to old: 0.1-0.8 for albumin and 0.05-0.5 for IgG), as measured using a semi-quantitative scale (0-3, higher score indicates more extravasation) ²⁹. The third study showed MMP-9 immunoreactivity in 30% of CAA affected vessels and in 79% of vessels with CMHs in old Tg2576 mice (n=4) while no MMP-9 immunoreactivity could be detected in young Tg2576 (n=2) or old wild type mice (n=2) ³³. The fourth study found evidence for BBB dysfunction in heterozygous TGF- β mice (n=5) with CMHs on histopathology in the form of a 12% increase in BBB permeability to a gadolinium-based contrast agent compared with wild type littermates (n=5) ³¹. In contrast, another MRI study did not find any difference in BBB leakage of gadolinium-based contrast material in transgenic arcA β mice with higher CMB frequencies (n=15) compared with wild type controls (n=15) ³⁰. The sixth study found no evidence for leakage of horseradish peroxidase or Trypan blue dye at sites beyond the vicinity of the hemorrhage in transgenic APP23 mice (n=3) and wild type littermates (n=3) ³².

Human studies

Of the 15 included studies, one assessed BBB dysfunction in HV-related ICH (n=82) ³⁴, six in CAA-related ICH (n=117) ³⁵⁻⁴⁰, and eight did not specify the underlying etiology of the ICH (n=435; table 2a, table 2b, and table e-4) ⁴¹⁻⁴⁸.

One study examined HV-related ICH patients *post mortem* (n=82) and reported endothelial integrity of cerebral arteries, arterioles and capillaries, and as such found no evidence for BBB dysfunction ³⁴.

Of six studies that assessed CAA-related BBB dysfunction, five studies found evidence for ICH-associated BBB dysfunction. Three studies assessed BBB abnormalities as defined by altered gelatinase levels in cortical vessels ^{35, 36}, cerebral tissue ³⁵, plasma ³⁵, and CSF ³⁷, in patients with CAA. The first study examined cortical vessels in detail with immunohistochemical staining, and identified an increased percentage of MMP-2 positive vessels in moderately to severely A β affected vessels from CAA patients (20-64% within and 62-100% around vessels with CAA grade 2-4; 95 vessels sampled from 6 cases) compared with not to mildly affected cortical vessels of the same patients (0-10% within and 0-27% around vessels with CAA grades 0-1; 90 vessels sampled from 6 cases) and cortical vessels of non-CAA controls (20% within and 0% around control vessels; 60 vessels sampled from 3 cases). No differences were found for MMP-9 ³⁵. In the same study MMP-2 and MMP-9 plasma concentrations were measured *in vivo* but their levels did not differ between CAA-related ICH (n=33) and non-CAA controls free of neurovascular and cardiovascular history (n=21). Besides, *post mortem* tissue pro-MMP-2 and pro-MMP-9 protein content was not different between individuals with CAA-related ICH (n=4) and non-CAA controls (n=3) ³⁵. A second study

found that 79% of CAA-affected vessels were immunopositive for MMP-9 (800 vessels per case, 6 CAA patients and 4 patients with CAA and Alzheimer disease (AD)), while only 5% of control vessels were immunopositive for MMP-9 (800 vessels per case, 4 non-CAA control cases)³⁶. MMP-9 immunoreactivity in the vessel wall was significantly increased in CAA vessels at late stages (stages 3 and 4) compared with early stages (stages 0-1), but no MMP-9 differences were observed between non-affected vessels of CAA patients and non-CAA controls³⁶. A third study assessed cerebrospinal fluid (CSF) levels of MMP-9, and tissue inhibitors of matrix metalloproteinases (TIMP-1 and TIMP-2) *in vivo*³⁷. Lower MMP-9 concentrations were found in CSF of individuals with AD or vascular dementia (VaD) with lobar CMBs (n=32) compared with AD or VaD patients without lobar CMBs (n=27) and cognitively normal controls (n=26). No group differences were observed for TIMP-1. TIMP-2 levels were elevated in AD/VaD without CMBs (n=27) compared with cognitively normal controls (n=26) and AD/VaD patients with CMBs (n=34)³⁷.

Two other studies examining CAA patients found evidence for BBB dysfunction. The first study in the form of decreased tight junction protein (TJP) occludin immunoreactivity in capillaries from the frontal cortex, and reduced levels of TJPs occludin and ZO-1 in leptomeningeal vessels as measured with Western blot analyses in patients with CAA (n=9) compared with non-CAA controls (n=10)³⁸. The second study used MRI to identify gadolinium-based contrast agent leakage through the BBB on T1-weighted images in a subset (n=2 out of 19, 11%) of individuals with CAA³⁹. In contrast, one study in patients with Alzheimer disease with (n=6) and without (n=12) lobar CMBs found no difference in p-glycoprotein expression⁴⁰.

Seven out of eight studies found evidence for BBB dysfunction without distinguishing between HV- and CAA-related ICH. Two of these found gadolinium-based contrast agent leakage on MRI in 48% to 85% of patients^{41, 42}. The first study reports a very mild increase in BBB permeability in the hemisphere contralateral to the ICH in 12 out of 25 patients 1 week after the first ICH symptoms occurred⁴¹, while the second study identified leakage of contrast agent into CSF, noncontiguous with the hematoma, in 39 out of 46 ICH patients within 5 days after symptom onset⁴². A third study used an iodine-based contrast agent to measure BBB dysfunction with computed tomography and found increased permeability in the hemisphere ipsilateral to the ICH (4.2+/-2.1 mL/100 mL per minute) relative to the contralateral hemisphere (3.7+/-1.6 mL/100 mL per minute; n=53) within 26 hours after symptom onset⁴³. No control group was included in any of these imaging studies. The fourth study described decreased immunoreactivity of TJP claudin-1 on visually inspected sections of basal ganglia tissue of patients with ICH (n=9) compared to sections of non-ICH controls (n=10)⁴⁴. The fifth study found increased CSF/serum albumin ratios in memory clinic patients with multiple CMBs (albumin ratio = 6.7, standard deviation (SD)

not reported; n=118), but not in memory clinic patients with single CMBs (albumin ratio=6.8, SD not reported; n=96) compared to those without CMBs (albumin ratio=6.1, SD not reported; n=825) ⁴⁵. The sixth study demonstrated increased vascular endothelial growth factor levels in serum of AD patients with CMBs (337+/-15 pg/mL; n=47) versus AD patients without CMBs (192+/-11pg/mL; n=99) ⁴⁶. The seventh study showed increased BBB dysfunction in the form of an increased CSF/serum albumin ratio in patients with cognitive decline with CMBs (8.3+/-1.9; n=15) compared with patients with cognitive decline but no CMBs (5.4+/-2.9; n=13) ⁴⁷. In contrast, the last study could not identify any difference in BBB dysfunction measured as CSF/serum albumin ratios between AD patients with CMBs (6.7+/-3; n=26) and AD patients without CMBs (7.4+/-4; n=26) ⁴⁸.

The effect size of the group difference between the studied sample and control sample of 5 human studies that quantified the mean (SD) of markers of BBB dysfunction is visualized in figure e-1. Three of the five studies report no difference in BBB dysfunction between patients and controls in these quantified measurements, which is in line with the results described in the previous paragraphs ^{35, 40, 48}. However, one of these studies did find a difference between patients and controls when using another measurement method which we could not include in our figure (percentage of MMP-2 positivity within and around vessels in moderately to severely A β affected vessels from ICH patients) ³⁵. Because of the large heterogeneity of markers used to assess BBB dysfunction and the differences in control samples that were used, we did not proceed to meta-analysis.

Table 1: Overview animal studies

Study	cSVD type	Hemorrhage type	Study design	Study sample (n)	Marker of BBB dysfunction	Main outcome	Evidence for BBB dysfunction
Lee et al., 2007 ²⁴	HV	ICH	L, P, IV	SP-SHR (7)	Leakage of contrast agent	Vascular permeability was increased in 42% of rats with ICH before ICH incidence	Yes
Bergerat et al., 2011 ²⁵	HV	ICH	CS, P, PM	Transgenic (hCETP) and non-transgenic stroke-prone Dahl-S rats (11)	Proteomic changes	Proteomic changes specific to increased stroke susceptibility profile are associated with BBB dysfunction	Yes
Jalal et al., 2012 ²⁷	HV	CMB	CS, P, PM	SP-SHR with UCAO and on a Japanese diet (5)	Plasma protein extravasation and altered gelatinase expression	IgG leakage and increased MMP-9 expression co-localized with CMBs	Yes
Schreiber et al., 2012 ²⁶	HV	CMB	CS, P, PM	SP-SHR (9)	Plasma protein deposition in vessel wall	CMBs occur for the first time several weeks after IgG vessel wall deposition can be detected	Yes
Elfenbein et al., 2007 ²⁸	CAA	CMB	CS, P, PM	Aged squirrel monkeys (4)	Plasma protein extravasation	Fibrinoid extravasation is apparent in animals with severe CAA and CMBs	Yes
Kumar-Singh et al., 2005 ²⁹	CAA	CMB	CS, P, PM	APP-sw transgenic mice (10) and PSAPP transgenic mice (n=6)	Plasma protein extravasation	Transgenic animals presented with more CMBs and with more albumin and IgG leakage	Yes
Lee et al., 2003 ³³	CAA	CMB	CS, P, PM	Transgenic APP-sw mice (4)	Altered gelatinase expression	MMP-9 expression was found in 30% of amyloid-laden vessels and in 79% of vessels with CMBs	Yes
Lifshitz et al., 2012 ³¹	CAA	CMB	CS, P, IV	Transgenic TGF- β 1 mice (5)	Leakage of contrast agent	12% increase in transgenic mice with CMBs compared with wild type mice	Yes
Klohs et al., 2013 ³⁰	CAA	CMB	L, P, IV	Transgenic arcA β mice (15)	Leakage of contrast agent	No difference between animals with and without CMBs	No
Winkler et al., 2001 ³²	CAA	ICH+CMB	CS, P, PM	Transgenic APP-23 mice (3)	HRP/Trypan blue leakage in cerebral tissue	No obvious leakage unless acute bleeding was present	No

Abbreviations: HV= hypertensive vasculopathy; CAA= cerebral amyloid angiopathy; ICH= intracerebral hemorrhage; CMB= cerebral microbleed; L= longitudinal; CS = cross-sectional; P=prospective; R=retrospective; n/a=not answerable; PM= *post mortem*; IV= *in vivo*; MMP= matrix metalloproteinase; SP-SHR = stroke-prone spontaneously hypertensive rats; IgG= immunoglobulin G; UCAO= unilateral carotid artery occlusion; Dahl-S = Dahl salt-sensitive; APP = amyloid precursor protein.

Table 2a: Overview human studies

Study	cSVD type	Determination cSVD type	Hemorrhage type	Study design	Study sample (n)	Marker of BBB dysfunction	Main outcome	Evidence for BBB dysfunction
Plesea et al., 2005 ³⁴	HV	Hypertensive patients	ICH	CS, n/a, PM	ICH patients (82)	Integrity of vascular endothelium	The endothelial structures of arteries, arterioles and capillaries were intact	No
Hernandez-Guillamon et al., 2012 ³⁵	CAA	Boston criteria ⁵¹	ICH	CS, P, PM, IV	CAA with ICH (6+33)	Altered gelatinase expression	MMP-2, but not MMP-9, was increased in and around CAA-affected vessels in ICH patients. No differences were found in plasma or in cerebral tissue	Yes
Zhao et al., 2015 ³⁶	CAA	PM pathologic examination	ICH+CMB	CS, P, PM	CAA with/without ICH (n=6), CAA with AD (n=4)	Altered gelatinase expression	The percentage of vessels with MMP-9 and MMP-9 expression were increased in CAA-affected vessels	Yes
Duits et al., 2015 ³⁷	CAA	Patients had lobar CMBs	CMB	CS, P, IV	AD/VaD with CMBs* (34)	Altered gelatinase expression	MMP-9 levels were decreased in CSF of individuals with AD with CMBs. TIMP-1 and TIMP-2 were similar to cognitively normal controls	Yes
Cheng et al., 2014 ³⁸	CAA	PM pathologic examination	CMB	CS, P, PM	CAA with CMBs (9)	Tight junction protein expression	Occludin expression was reduced in leptomeningeal and cerebral vessels	Yes

Study	cSVD type	Determination cSVD type	Hemorrhage type	Study design	Study sample (n)	Marker of BBB dysfunction	Main outcome	Evidence for BBB dysfunction
Hartz et al., 2012 ³⁹	CAA	Boston criteria ⁵¹	ICH+CMB	CS, P, <i>IV</i>	CAA with ICH or CMBs (19)	Leakage of contrast agent	Contrast agent leakage was apparent in 11% of ICH cases	Yes
Van Assema et al., 2012 ⁴⁰	CAA	Patients had lobar CMBs	CMB	CS, P, <i>IV</i>	AD with lobar CMBs (6)	Altered pgp expression	Pgp expression was not different between AD patients with and without CMBs	No

* Study samples partly overlap for these studies. Abbreviations: cSVD=cerebral small vessel disease; BBB=blood-brain barrier; HV= hypertensive vasculopathy; CAA= cerebral amyloid angiopathy; ICH= intracerebral hemorrhage; CMB= cerebral microbleed; L= longitudinal; CS = cross-sectional; P=prospective; R=retrospective; n/a=not answerable; *PM*= *post mortem*; *IV*= *in vivo*; MMP= matrix metalloproteinase; AD= Alzheimer disease; VaD=vascular dementia; TIMP= tissue inhibitor of metalloproteinase; CSF= cerebrospinal fluid.

Table 2b: Overview human studies (continued from table 2a)

Study	cSVD type	Determination cSVD type	Hemorrhage type	Study design	Study sample (n)	Marker of BBB dysfunction	Main outcome	Evidence for BBB dysfunction
Aksoy et al., 2013 ⁴¹	40% HV, 20% CAA, 40% other	n/a	ICH	CS, P, IV	ICH patients (25)	Leakage of contrast agent	Contrast agent leakage was apparent in contralateral hemisphere in 48% of ICH cases	Yes
Kidwell et al., 2011 ⁴²	Unspecific d, primary ICH	n/a	ICH	CS, R, IV	ICH patients (46)	Leakage of contrast agent	Contrast agent leakage was apparent in CSF in 85% of ICH cases	Yes
McCourt et al., 2015 ⁴³	Unspecific d, primary ICH	n/a	ICH	CS, P, IV	ICH patients (53)	Leakage of contrast agent	Contrast agent leakage was apparent in ipsilateral hemisphere compared with contralateral hemisphere	Yes
Zhang et al., 2016 ⁴⁶	Unspecific d	n/a	CMB	CS, P, IV	AD with CMBs (47)	Serum VEGF levels	Serum VEGF levels were increased in AD patients with CMBs	Yes
Shams et al., 2016 ⁴⁵	Unspecific d	n/a	CMB	CS, P, IV	Memory clinic patients with CMBs (214, of which 118 with multiple CMBs)	Altered CSF/serum albumin ratio	Albumin ratio was increased in memory clinic patients with multiple CMBs	Yes
Poliakova et al., 2016 ⁴⁷	Unspecific d	n/a	CMB	CS, P, IV	Patients with cognitive decline with CMBs (15)	Altered CSF/serum albumin ratio	Albumin ratio was increased in patients with cognitive decline with CMBs	Yes

Study	cSVD type	Determination cSVD type	Hemorrhage e type	Study design	Study sample (n)	Marker of BBB dysfunction	Main outcome	Evidence for BBB dysfunction
Tran et al., 2016 ⁴⁴	Unspecific d	n/a	ICH+CMB	CS, R, <i>PM</i>	ICH patients (9)	Tight junction protein expression	Claudin-1 expression was decreased in cerebral vessels in ICH cases	Yes
Goos et al., 2012 ⁴⁸	Unspecific d	n/a	CMB	CS, P, <i>IV</i>	AD with CMBs* (26)	Altered CSF/serum albumin ratio	Albumin ratio was not different between AD patients with and without CMBs	No

* Study samples partly overlap for these studies. Abbreviations: cSVD=cerebral small vessel disease; BBB=blood-brain barrier; HV= hypertensive vasculopathy; CAA= cerebral amyloid angiopathy; ICH= intracerebral hemorrhage; CMB= cerebral microbleed; L= longitudinal; CS = cross-sectional; P=prospective; R=retrospective; n/a=not answerable; *PM*= *post mortem*; *IV*= *in vivo*; AD= Alzheimer disease; CSF= cerebrospinal fluid; VEGF= vascular endothelial growth factor

DISCUSSION

In this systematic review we found support for a possible role of BBB dysfunction in the pathophysiology of cSVD-related ICH and CMBs/CMHs in 20 out of 25 studies. We found BBB dysfunction in both animal and human studies, and in HV-related ICH or CMBs/CMHs and CAA-related ICH or CMBs/CMHs. Signs for BBB disruption were found in studies with *in vivo* BBB assessment, including MRI, CT and CSF/serum analysis, as well as in studies using *post mortem* histopathological or proteomic analyses to assess BBB dysfunction. Animal studies suggest that BBB dysfunction precedes ICH or CMB/CMH occurrence, but it remains unclear whether this relationship is causal.

Leakage of contrast agents and plasma proteins in the brain parenchyma or CSF as well as increased CSF/serum albumin ratios indicate general BBB dysfunction across studies in HV-related ICH²⁴ and CMBs/CMHs²⁷, CAA-related ICH^{29, 39} and CMBs/CMHs^{28, 29, 31, 32}, and those with unspecified or mixed pathology^{41-43, 45, 47}. Based on the available observations in the included studies, we postulate that multiple pathways may contribute to BBB dysfunction, which may in part be caused by the underlying pathology. In hypertensive animal models with CMHs, leakage of plasma components into the vessel wall²⁶ and increased MMP-9 expression²⁷ suggest that degradation of the basement membrane and plasma protein deposition contribute to vessel wall instability. In a hypertensive animal model of ICH, pre-stroke proteomic vessel changes also provide signs indicating an altered basement membrane composition²⁵. These vessel changes in HV may interfere with the vessels' autoregulatory ability to respond to changes in blood pressure, which may in turn predispose to rupture in the context of hypertension. The inner endothelial layer was intact in humans with HV-related ICH³⁴ and as such may not be affected until the moment of the actual rupture.

In CAA, tight junction protein expression was reduced in humans³⁸, and altered MMP expression was found in an animal model³³ and in humans³⁵⁻³⁷. These findings suggest that MMP-mediated degradation of the vascular basement membrane and tight junction protein dysfunction may be mechanisms in the formation of CAA-related ICH or CMBs/CMHs. These BBB abnormalities, together with A β accumulation in the vessel wall, may weaken the vessel wall, interfere with its ability to autoregulate, and make it prone to rupture. It remains unclear from the included studies whether BBB abnormalities lead to A β deposition/clearance abnormalities, or whether the A β deposition precedes the BBB dysfunction.

Studies that did not discriminate between CAA or HV describe decreased tight junction protein expression in ICH patients⁴⁴ and increased serum VEGF levels in AD patients with CMBs

⁴⁶. The latter finding suggests that angiogenesis with formation of vessels with BBB dysfunction may play a role in CMB formation.

Two of the three human studies that did not find indications for BBB dysfunction compared AD patients with CMBs to AD patients without CMBs ^{40, 48}. A possible explanation for the fact that these studies found no evidence for BBB dysfunction in relation to presence of CMBs is latent presence of HV and CAA in AD without CMBs, such that the control AD group also has BBB dysfunction to some degree. Methodological issues such as low sensitivity of the marker of BBB dysfunction and statistically underpowered analyses due to restricted sample size could also have played a role. Other studies included in this review that compared AD patients or memory clinic patients with and without CMBs did find increased BBB dysfunction in relation to CMBs ^{37, 45-47}.

Animal studies are considered a valuable tool to study the temporal relationship of pathophysiological processes. However, the majority of animal studies included in this review did not have a longitudinal but a cross-sectional design. Most of the cross-sectional studies found BBB abnormalities in concurrence with the presence of CMHs ^{27-29, 31-33}, but some observed BBB changes in animal models of HV or CAA before they reached the age at which hemorrhages typically occur ^{25, 26}. Only two studies assessed the relation between BBB dysfunction and occurrence of CMBs or ICH longitudinally. One study did not identify BBB alterations in relation to CMBs ³⁰, while the other study was able to monitor ICH development and preceding co-localized BBB dysfunction *in vivo* ²⁴. A recent case-report of a patient with probable CAA showed development of a new CMB exactly at the location of gadolinium leakage on 7T MRI ¹⁰. These findings, although the evidence is limited, suggest that BBB dysfunction may precede cerebral hemorrhage.

Strengths of this review include the systematic literature search and inclusion of both human and animal studies, which allowed us to summarize the evidence of BBB dysfunction assessed by a variety of methods and in multiple and variable cohorts of cSVD related ICH patients and animals. In addition, the inclusion of animal studies allowed interpretations in terms of disease mechanisms and could provide a first indication of the temporal relationship between BBB dysfunction and cerebral hemorrhage.

Our systematic review also has limitations. Many of the included studies had a relatively small sample size, seven studies lacked a control group, and results were not quantified in five studies. Some of the CMBs rated on MRI, especially beyond the context of CAA, may correspond to other lesion types such as microinfarcts with a hemorrhagic component, which may bias the results ⁴⁹. Furthermore, caution is warranted since negative findings in general and presence of hemorrhage in cSVD animal models are likely to be underreported, leading to publication bias and selective reporting within studies. Moreover, results on various transgenic animal models with CAA were

reported but these models may not be specific to cSVD since the animals also exhibit AD pathology, and several studies examined animals at a relatively young age. Although the findings in animal models are valuable for understanding disease physiology, it should be kept in mind that these models do not perfectly mimic human disease. While the human studies suggest possible involvement of BBB dysfunction in ICH or CMB formation, their cross-sectional nature hampers the ability of making any causal inferences. We only identified one human study that assessed HV-related ICH, so future studies on this topic are warranted. Although we reported differences in study quality according to standard assessment scales, we cannot compare the strength or specificity of each individual BBB assessment approach. As a result, and because of the wide variety of the included animal models and study samples, it is difficult to extrapolate the findings in this review outside the context of each individual study. Finally, although previous work has suggested that CMBs are predictors of future ICH¹⁸, it is not yet clear whether these two types of hemorrhage constitute similar pathophysiological foundations⁵⁰.

To increase insights into the role of BBB dysfunction in the pathophysiology of ICH and CMBs/CMHs, dedicated prospective studies of animal models of CAA and HV with serial *in vivo* neuroimaging examinations combined with *post mortem* BBB assessment are needed. This should be combined with longitudinal *in vivo* monitoring of BBB integrity in various cohorts and *post mortem* assessments in humans. Such studies are, unfortunately, hard to establish because it will be difficult to achieve a reasonable sample size. The development of more sensitive methods to measure and localize BBB dysfunction *in vivo* may identify individuals at risk for ICH. Importantly, supporting BBB integrity may be a potential target in developing strategies to prevent ICH and CMBs/CMHs in the context of cSVD, and monitoring BBB dysfunction could be a method to evaluate the efficiency of treatment strategies.

CONCLUSION

This systematic review suggests that BBB dysfunction plays a role in the pathophysiology of cSVD-related ICH and CMBs/CMHs. The hypothesis that BBB dysfunction precedes ICH or CMB/CMH formation needs confirmation in larger longitudinal studies in humans.

REFERENCES

1. Krishnamurthi RV, Feigin VL, Forouzanfar MH, et al. Global and regional burden of first-ever ischaemic and haemorrhagic stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. *The Lancet Global health* 2013;1:e259-281.
2. van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. *The Lancet Neurology* 2010;9:167-176.
3. Poon MT, Fonville AF, Al-Shahi Salman R. Long-term prognosis after intracerebral haemorrhage: systematic review and meta-analysis. *Journal of neurology, neurosurgery, and psychiatry* 2014;85:660-667.
4. Jolink WM, Klijn CJ, Brouwers PJ, Kappelle LJ, Vaartjes I. Time trends in incidence, case fatality, and mortality of intracerebral hemorrhage. *Neurology* 2015;85:1318-1324.
5. Grinberg LT, Thal DR. Vascular pathology in the aged human brain. *Acta Neuropathol* 2010;119:277-290.
6. Sutherland GR, Auer RN. Primary intracerebral hemorrhage. *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia* 2006;13:511-517.
7. Vinters HV. Cerebral amyloid angiopathy. A critical review. *Stroke* 1987;18:311-324.
8. Charidimou A, Boulouis G, Gurol ME, et al. Emerging concepts in sporadic cerebral amyloid angiopathy. *Brain : a journal of neurology* 2017;140:1829-1850.
9. Fisher M, French S, Ji P, Kim RC. Cerebral microbleeds in the elderly: a pathological analysis. *Stroke* 2010;41:2782-2785.
10. van Nieuwenhuizen KM, Hendrikse J, Klijn CJM. New microbleed after blood-brain barrier leakage in intracerebral haemorrhage. *BMJ Case Rep* 2017;2017.
11. Ballabh P, Braun A, Nedergaard M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis* 2004;16:1-13.
12. Sweeney MD, Ayyadurai S, Zlokovic BV. Pericytes of the neurovascular unit: key functions and signaling pathways. *Nat Neurosci* 2016;19:771-783.
13. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nature reviews Neuroscience* 2006;7:41-53.
14. Abbott NJ. Blood-brain barrier structure and function and the challenges for CNS drug delivery. *Journal of inherited metabolic disease* 2013;36:437-449.
15. Wardlaw JM. Blood-brain barrier and cerebral small vessel disease. *J Neurol Sci* 2010;299:66-71.
16. Carrano A, Hoozemans JJ, van der Vies SM, van Horssen J, de Vries HE, Rozemuller AJ. Neuroinflammation and blood-brain barrier changes in capillary amyloid angiopathy. *Neurodegenerative diseases* 2012;10:329-331.
17. Greenberg SM, Vernooij MW, Cordonnier C, et al. Cerebral microbleeds: a guide to detection and interpretation. *The Lancet Neurology* 2009;8:165-174.
18. Greenberg SM, Eng JA, Ning M, Smith EE, Rosand J. Hemorrhage burden predicts recurrent intracerebral hemorrhage after lobar hemorrhage. *Stroke* 2004;35:1415-1420.
19. Charidimou A, Werring DJ. Cerebral microbleeds as a predictor of macrobleeds: what is the evidence? *International journal of stroke : official journal of the International Stroke Society* 2014;9:457-459.
20. Fazekas F, Kleinert R, Roob G, et al. Histopathologic analysis of foci of signal loss on gradient-echo T2-weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds. *Ajnr: American Journal of Neuroradiology* 1999;20:637-642.
21. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm. Accessed March 30, 2017.
22. Herzog R, Alvarez-Pasquin MJ, Diaz C, Del Barrio JL, Estrada JM, Gil A. Are healthcare workers' intentions to vaccinate related to their knowledge, beliefs and attitudes? A systematic review. *BMC public health* 2013;13:154.
23. Sena E, van der Worp HB, Howells D, Macleod M. How can we improve the pre-clinical development of drugs for stroke? *Trends in neurosciences* 2007;30:433-439.
24. Lee JM, Zhai G, Liu Q, et al. Vascular permeability precedes spontaneous intracerebral hemorrhage in stroke-prone spontaneously hypertensive rats. *Stroke* 2007;38:3289-3291.

25. Bergerat A, Decano J, Wu CJ, et al. Prestroke proteomic changes in cerebral microvessels in stroke-prone, transgenic[hCETP]-Hyperlipidemic, Dahl salt-sensitive hypertensive rats. *Molecular Medicine* 2011;17:588-598.
26. Schreiber S, Bueche CZ, Garz C, et al. The pathologic cascade of cerebrovascular lesions in SHRSP: Is erythrocyte accumulation an early phase. *Journal of Cerebral Blood Flow and Metabolism* 2012;32:278-290.
27. Jalal FY, Yang Y, Thompson J, Lopez AC, Rosenberg GA. Myelin loss associated with neuroinflammation in hypertensive rats. *Stroke* 2012;43:1115-1122.
28. Elfenbein HA, Rosen RF, Stephens SL, et al. Cerebral beta-amyloid angiopathy in aged squirrel monkeys. *Histology & Histopathology* 2007;22:155-167.
29. Kumar-Singh S, Pirici D, McGowan E, et al. Dense-core plaques in Tg2576 and PSAPP mouse models of Alzheimer's disease are centered on vessel walls. *The American journal of pathology* 2005;167:527-543.
30. Klohs J, Politano IW, Deistung A, et al. Longitudinal Assessment of Amyloid Pathology in Transgenic ArcAbeta Mice Using Multi-Parametric Magnetic Resonance Imaging. *PloS one* 2013;8.
31. Lifshitz V, Weiss R, Benromano T, et al. Immunotherapy of cerebrovascular amyloidosis in a transgenic mouse model. *Neurobiology of aging* 2012;33:432.e431-432.e413.
32. Winkler DT, Bondolfi L, Herzig MC, et al. Spontaneous hemorrhagic stroke in a mouse model of cerebral amyloid angiopathy. *Journal of Neuroscience* 2001;21:1619-1627.
33. Lee JM, Yin KJ, Hsin I, et al. Matrix metalloproteinase-9 and spontaneous hemorrhage in an animal model of cerebral amyloid angiopathy. *Annals of neurology* 2003;54:379-382.
34. Plesea IE, Camenita A, Georgescu CC, et al. Study of cerebral vascular structures in hypertensive intracerebral haemorrhage. *Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie* 2005;46:249-256.
35. Hernandez-Guillamon M, Martinez-Saez E, Delgado P, et al. MMP-2/MMP-9 plasma level and brain expression in cerebral amyloid angiopathy-associated hemorrhagic stroke. *Brain Pathology* 2012;22:133-141.
36. Zhao L, Arbel-Ornath M, Wang X, et al. Matrix metalloproteinase 9-mediated intracerebral hemorrhage induced by cerebral amyloid angiopathy. *Neurobiology of aging* 2015;36:2963-2971.
37. Duits FH, Hernandez-Guillamon M, Montaner J, et al. Matrix Metalloproteinases in Alzheimer's Disease and Concurrent Cerebral Microbleeds. *Journal of Alzheimer's Disease* 2015;48:711-720.
38. Cheng X, He P, Yao H, Dong Q, Li R, Shen Y. Occludin deficiency with BACE1 elevation in cerebral amyloid angiopathy. *Neurology* 2014;82:1707-1715.
39. Hartz AM, Bauer B, Soldner EL, et al. Amyloid-beta contributes to blood-brain barrier leakage in transgenic human amyloid precursor protein mice and in humans with cerebral amyloid angiopathy. *Stroke* 2012;43:514-523.
40. van Assema DM, Goos JD, van der Flier WM, et al. No evidence for additional blood-brain barrier P-glycoprotein dysfunction in Alzheimer's disease patients with microbleeds. *Journal of Cerebral Blood Flow & Metabolism* 2012;32:1468-1471.
41. Aksoy D, Bammer R, Mlynash M, et al. Magnetic resonance imaging profile of blood-brain barrier injury in patients with acute intracerebral hemorrhage. *Journal of the American Heart Association* 2013;2:e000161.
42. Kidwell CS, Burgess R, Menon R, Warach S, Latour LL. Hyperacute injury marker (HARM) in primary hemorrhage: A distinct form of CNS barrier disruption. *Neurology* 2011;77:1725-1728.
43. McCourt R, Gould B, Kate M, et al. Blood-brain barrier compromise does not predict perihematoma edema growth in intracerebral hemorrhage. *Stroke* 2015;46:954-960.
44. Tran KA, Zhang X, Predescu D, et al. Endothelial beta-Catenin Signaling Is Required for Maintaining Adult Blood-Brain Barrier Integrity and Central Nervous System Homeostasis. *Circulation* 2016;133:177-186.
45. Shams S, Granberg T, Martola J, et al. Cerebrospinal fluid profiles with increasing number of cerebral microbleeds in a continuum of cognitive impairment. *Journal of Cerebral Blood Flow and Metabolism* 2016;36:621-628.
46. Zhang JB, Li MF, Zhang HX, et al. Association of serum vascular endothelial growth factor levels and cerebral microbleeds in patients with Alzheimer's disease. *European journal of neurology : the official journal of the European Federation of Neurological Societies* 2016.

47. Poliakova T, Levin O, Arablinskiy A, Vasenina E, Zerr I. Cerebral microbleeds in early Alzheimer's disease. *Journal of Neurology* 2016;263:1961-1968.
48. Goos JD, Teunissen CE, Veerhuis R, et al. Microbleeds relate to altered amyloid-beta metabolism in Alzheimer's disease. *Neurobiology of aging* 2012;33:1011.e1011-1019.
49. van Veluw SJ, Charidimou A, van der Kouwe AJ, et al. Microbleed and microinfarct detection in amyloid angiopathy: a high-resolution MRI-histopathology study. *Brain : a journal of neurology* 2016;139:3151-3162.
50. Greenberg SM, Nandigam RN, Delgado P, et al. Microbleeds versus macrobleeds: evidence for distinct entities. *Stroke* 2009;40:2382-2386.
51. Knudsen KA, Rosand J, Karluk D, Greenberg SM. Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. *Neurology* 2001;56:537-539.

SUPPLEMENTARY MATERIAL

Appendix e-1

Search strategy implemented in OVID MEDLINE and EMBASE

- 1) exp blood-brain barrier/
- 2) Blood-brain barrier.tw.
- 3) exp capillary permeability/and exp brain/
- 4) exp microcirculation/and exp brain/
- 5) exp endothelium, vascular/and exp brain/
- 6) exp tight junction/and exp brain/
- 7) (capillary permeability adj10 (cerebral or brain)).tw.
- 8) (vascular permeability adj10 (cerebral or brain)).tw
- 9) (microcirculation adj10 (cerebral or brain)).tw.
- 10) (vascular endothelium adj10 (cerebral or brain)).tw
- 11) (tight junction adj10 (cerebral or brain)).tw.
- 12) (blood adj5 vessel adj5 wall).tw.
- 13) BBB.tw
- 14) or/1-13
- 15) exp intracerebral h?emorrhage/
- 16) (h?emorrhag\$ adj5 (cerebral or brain or intracerebral or intraparenchymal or intracranial)).tw.
- 17) (h?ematoma adj5 (cerebral or brain or intracerebral or intraparenchymal or intracranial)).tw.
- 18) (bleeding adj5 (cerebral or brain or intracerebral or intraparenchymal or intracranial)).tw.
- 19) (microbleed\$ adj5 (cerebral or brain or intracerebral or intraparenchymal or intracranial)).tw.
- 20) (microh?emorrhag\$ adj5 (cerebral or brain or intracerebral or intraparenchymal or intracranial)).tw.
- 21) microbleed\$.tw.
- 22) microh?emorrhag\$.tw.
- 23) or/15-22
- 24) 14 and 23
- 25) limit 24 to review
- 26) 24 not 25

Table e-1: Quality assessment Human Studies according to Newcastle-Ottawa scale ^{21, 22}

Selection				Comparability			Outcome/Exposure		
Study type	Representativeness of the sample	Sample size	Non-respondents	Ascertainment of exposure	Study controls (most important factor)	Study controls for (additional factor)	Assessment of outcome	Statistical test	Score total
Plessea et al., 2005 ³⁴	0	0	n/a	2	0	0	0		2/9
Hartz et al., 2012 ³⁹	1 (consecutive series of cases)	0	0	2	0	0	2	1	6/10
Aksoy et al., 2013 ⁴¹	0	0	0	2	0	0	2	1	5/10
Kidwell et al., 2011 ⁴²	1 (consecutive series of cases)	1 (explorative study)	0	2	1 (MRI timing)	1 (contrast dosage)	2	1	9/10
McCourt et al., 2015 ⁴³	1 (random sampling)	1 (n>50)	0	2	1 (hypertension)	0	2	1	8/10
Hernandez-Guillamon et al., 2012 part 1 (<i>in vivo</i>) ³⁵	1 (MRI)	1 (consecutive series of cases)	1	0	0	0	1	1	6/9
Hernandez-Guillamon et al., 2012 part 2 + 3 (<i>post-mortem</i>) ³⁵	1 (pathologic confirmation)	0	1	0	0	0	1	1	5/9

Selection			Comparability				Outcome/Exposure		
Study type	Is the case definition adequate?	Representativeness of cases	Selection of Controls	Definition of controls	Study controls for (most important factor)	Study controls for (additional factor)	Ascertainment of exposure for cases and controls	Non-response rate	Score total
Cheng et al., 2014 ³⁸	Case-control 1 (iron stain)	1 (random sample)	1	0	1 (age-matched)	1 (matched for sex, post mortem interval, and sample preparations) 1 (sex-matched)	1	1	8/9
Duits et al., 2015 ³⁷	Case-control 1 (MRI)	0	1	1	1 (age-matched)	1 (sex-matched)	1	0 (missing data)	7/9
Zhao et al., 2015 ³⁶	Case-control 0 (only symptomatic haemorrhages rated)	0	1	0	1 (age-matched)	0	1	1	5/9
Van Assema et al., 2012 ⁴⁰	Case-control 1 (MRI)	0	1	1	0	0	1	1	6/9
Zhang et al., 2016 ⁴⁶	Case-control 1 (MRI)	1 (consecutive series of cases)	1	1	1 (age-adjusted analysis)	1 (sex, cardiovascular risk factors, lacunar infarction, WMH adjusted analyses)	1	1	9/9
Shams et al., 2016 ⁴⁵	Case-control 1 (MRI)	1 (consecutive series of cases)	1	1	0	0	1	1	7/9
Poliakova et al., 2016 ⁴⁷	Case-control 1 (MRI)	0	0	1	0	0	1	0 (missing data)	4/9
Goos et al., 2012 ⁴⁸	Case-control 1 (MRI)	0	1	1	1 (Age-matched)	1 (sex-matched)	1	0 (missing data)	7/9
Tran et al., 2016 ⁴⁴	Case-control 1 (symptoms and H&E)	0	1	1	0	0	1	1	6/9

Abbreviation key: n/a, not applicable; MRI, magnetic resonance imaging; WMH, white matter hyperintensities; H&E, hematoxylin and eosin.

Abbreviation key: n/a, not applicable; MRI, magnetic resonance imaging; WMH, white matter hyperintensities; H&E, hematoxylin and eosin.

Table e-2: Quality assessment animal studies according to CAMARADES scale ²³

	Publication in peer- reviewed journal	Statement of control of temperature	Randomization of treatment or control	Allocation concealment	Blinded assessment of outcome	Type of anesthetics specified *	Use of animals with hypertension or CAA*	Sample- size calculation	Statement of compliance with regulatory requirements	Statement regarding possible conflict of interest	Score total
Lee et al., 2007 ²⁴	1	0	n/a	n/a	n/a	1 (isoflurane during MRI)	1	0	1	1	5/7
Bergerat et al., 2011 ²⁵	1	0	n/a	n/a	n/a	0 (general anesthesia)	1	0	1	1	4/7
Jalal et al., 2012 ²⁷	1	1	n/a	n/a	n/a	1 (2% isoflurane during surgery/blood collection and 50mg/kg pentobarbital before tissue collection)	1	0	1	1	6/7
Schreiber et al., 2012 ²⁶	1	0	n/a	n/a	n/a	1 (1%-1.5% isoflurane during MRI)	1	0	1	1	4/7
Winkler et al., 2001 ³²	1	0	n/a	n/a	n/a	1 (pentobarbital overdose before tissue collection)	1	0	0	1	4/7
Elfenbein et al., 2007 ²⁸	1	0	n/a	n/a	n/a	1 (15mg/kg ketamine and 100mg/kg pentobarbital before tissue collection)	1	0	1	1	5/7

Publication in peer- reviewed journal	Statement of control of temperature	Randomization of treatment or control	Allocation concealment	Blinded assessment of outcome	Type of anesthetics specified *	Use of animals with hypertension or CAA*	Sample- size calculation	Statement of compliance with regulatory requirements	Statement regarding possible conflict of interest	Score total
Klohs et al., 2013 ³⁰	1	n/a	n/a	n/a	1 (3% isoflurane before MRI, 1.5% isoflurane during DCE-MRI)	1	0	1	1	6/7
Kumar-Singh et al., 2005 ²⁹	0	n/a	n/a	n/a	n/a (animals were euthanized by cervical dislocation)	1	0	1	1	4/6
Lee et al., 2003 ³³	0	n/a	n/a	n/a	0 (not mentioned in text)	1	0	0	1	3/7
Lifshitz et al., 2012 ³¹	0	n/a	n/a	n/a	1 (1-3% isoflurane during MRI)	1	0	1	1	5/7

* Item adjusted from original CAMARADES scale²³ to better reflect study quality of non-drug trial studies in hypertension and CAA animal models

Abbreviation key: MRI, magnetic resonance imaging; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; CAA, cerebral amyloid angiopathy; n/a, not applicable.

Table e-3: Methodology in animal studies

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessment method	Location	BBB dysfunction marker	Reason authors chose this marker	Assessment method BBB dysfunction	Mean(sd) of BBB dysfunction study sample	Mean(sd) of BBB dysfunction control sample	Contrast	Study sample size	Study sample age	Study sample male	Control sample size	Control sample age	Control sample male
Lee et al., 2007 ²⁴	ICH symptoms / MRI (70%)	T2*-weighted images	Cortex, corpus callosum, striatum	CE lesions	Contrast agent extravasates when BBB dysfunctional	DCE-MRI	42%	n/a	Absent	7	n/a	100%	n/a	n/a	n/a
Berge et al., 2011 ²⁵	Earlier knowledge on animal model (n/a)	n/a	n/a	Expression of various proteins	Proteins have been reported in literature to be part of BBB, and their increase is expected to alter BBB function	Immunohistochemistry and QSPEC analysis	Aquaporin-4, G-protein $\alpha 12$, Collagen XII, Carbonic anhydrase-4, NHE-3-regulator-2, syntrophin $\alpha 1$, Filamin B, Transferrin, Laminin $\alpha 1$	n/a	Young non-transgenic dahl-s rats	11	4.5 months	27	15	2-4.5 months	60

Study	Henorrhage marker (% study sample with hemorrhage)	Assessment method hemorrhage	Location hemorrhage	BBB dysfunction marker	Reason authors chose this marker	Assessment method BBB dysfunction	Mean(s) marker of BBB dysfunction study sample	Mean(s) marker of BBB dysfunction control sample	Control sample	Study sample size	Study sample age	Study sample male	Control sample size	Control sample age	Control sample male
Jalal et al., 2012 ²⁷	Microscope (n/a)	H&E	100% white matter	MMP-2, MMP-3, MMP-9, IgG immunoreactivity	IgG extravasates when BBB disrupted, MMPs have been associated with BBB disruption	Immunoblotting & immunohistochemistry	n/a	n/a	Sham operated SP-SHR on a regular diet	5	16-17 weeks	100	5	16-17 weeks	100
Schreiber et al., 2012 ²⁶	Microscope, MRI (n/a)	H&E, T2-weighted images	Basal ganglia, cortex, hippocampus, corpus callosum	IgG immunoreactivity	IgG extravasates when BBB is disrupted	Immunohistochemistry	n/a	n/a	Abse- nt	9	18-36 weeks	100	n/a	n/a	n/a
Elfenbein et al., 2007 ²⁸	Microscope (n/a)	DAB-enhanced Perls' method	n/a	Eosinophilic fibrinoid	Fibrin extravasates when BBB is disrupted	H&E	n/a	n/a	Young squirrel monkey	4	20	100	1	7	100

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessment method	Location hemorrhage	BBB dysfunction marker	Reason authors chose this marker	Assessment method BBB dysfunction	Mean(sd) marker of BBB dysfunction study sample	Mean(sd) marker of BBB dysfunction control sample	Control sample	Study sample size	Study sample age	Study sample % male	Control sample size	Control sample age	Control sample % male
Lee et al., 2003 ³³	Microscope (n/a)	Perls' blue	n/a	Vessels /CMBs positive for MMP-9 immunoreactivity	MMPs have been associated with BBB disruption	Immunofluorescence	30% of CAA affected vessels and 79% of CMBs	0% immunoreactivity	Wild type mice + young APPs wild mice	4	15-18 hs	n/a	2+2	24 months + 3 months	n/a
Lifshitz et al., 2012 ³¹	Microscope (n/a)	Perls' blue	n/a	T1-weighted image enhancement due to gadolinium leakage	Contrast agent extravasates when BBB dysfunctional	Pre-post contrast MRI	12% signal enhancement	n/a	Wild type mice	5	16 months	n/a	5	16 months	n/a
Klohs et al., 2013 ³⁰	MRI (n/a)	SWI	Predominantly lobar	Median k-trans value within ROI (gadolinium leakage rate)	Contrast agent extravasates when BBB dysfunctional	DCE-MRI	n/a	n/a	Wild type mice	15	n.a	n/a	15	n/a	n/a

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessment method hemorrhage	Location hemorrhage	BBB dysfunction marker	Reason authors chose this marker	Assessment method BBB dysfunction	Mean(sd) marker of BBB dysfunction study sample	Mean(sd) marker of BBB dysfunction control sample	Control sample	Study sample size	Study sample age	Study sample size	Study sample age	Study sample size	Study sample age	Study sample size	Study sample age	Study sample size	Study sample age
Winkler et al., 2001 ³²	Microscope (n/a)	H&E, Perl's blue	Neocortex, thalamus, and hippocampus	BBB leakage of HRP/Trypan blue	HRP/Trypan blue extravasate when BBB dysfunctional	Immunohistochemistry, confocal microscopy	n/a	n/a	Littermate control mice	3	24 months	3	24 months	3	24 months	3	24 months	3	n/a

Abbreviation key: BBB = blood-brain barrier; sd = standard deviation; DCE-MRI = dynamic contrast enhanced magnetic resonance imaging; CE = contrast enhancement; ICH = intracerebral hemorrhage; CAA = cerebral amyloid angiopathy; APPsw, transgenic mouse model expressing the Swedish mutation of the human amyloid precursor protein; DAB, 3, 3'-H&E = hematoxylin and eosin; MMP = matrix metalloproteinase; CMB = cerebral microbleed; SP-SHR = stroke-prone spontaneously hypertensive rats; SWI = susceptibility weighted imaging; IgG = immunoglobulin G; HRP = horseradish peroxidase; ROI = region of interest

Table e-4: methodology in human studies

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessment method hemorrhage	Location hemorrhage	BBB dysfunction marker	Reason author chose this marker	Assessment method BBB dysfunction	Mean(s) marker of BBB dysfunction study sample	Mean(s) marker of BBB dysfunction control sample	Control sample	Study sample	Control sample	Size	Age	Sex	Sample
Plesea et al., 2005 ³⁴	ICH symptoms/pathologic evaluation (100%)	H&E	n/a	CD-34 immunoreactivity	Structural integrity ECs reflects BBB integrity	Immunohistochemistry	n/a	n/a	Absent	82	82	n/a	n/a	n/a	n/a
Heman dez-Guilla mon et al., 2012 part 1 ³⁵	MRI (100%)	T2*-weighted images	100% lobar	MMP-2 and MMP-9 precursor concentrations in plasma	MMPs have been associated with BBB dysfunction	ELISA	MMP-2, 1459.29 +/- 330.58 ng/mL; MMP-9 77.95 +/- 53.56 ng/mL	MMP-2, 1458.99 +/- 355.25 ng/mL; MMP-9 66.54 +/- 38.10 ng/mL	Controls free of neurovascular and cardiovascular history	33	33	n/a	61	21	n/a
Heman dez-Guilla mon et al., 2012 part 2 ³⁵	ICH symptoms/pathologic evaluation (100%)	n/a	n/a	MMP-2 and MMP-9 immunoreactivity in cortical tissue	MMPs have been associated with BBB dysfunction	Immunoblotting/immunofluorescence	n/a	n/a	Controls that died without CAA and ICH	4	4	85	25	3	76

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessm ent method hemorrh age	Location hemorrh age	BBB dysfunction marker	Reason authors chose this marker	Assessment method BBB dysfunction	Mean(sd) of BBB dysfunction study sample	Mean(sd) marker of BBB dysfunction control sample	Contr ol sample	Study sample size	ag e	% male	Study sample size	ag e	% male	Control sample size	ag e	% male
Hemandez-Guillamon et al., 2012 part 3 ³⁵	ICH symptoms/pathologic evaluation (100%)	n/a	n/a	% of MMP-2 and MMP-9 positive vessels	MMPs have been associated with BBB dysfunction	immunohistochemistry	20% within and 62% around CAA grade 2 vessels (n=60), 64% within and 100% around CAA grade 3 vessels (n=11), 50% within and 100% around CAA grade 4 vessels (n=24)	(10% within and 0% around CAA grade 0 vessels (n=30), 0% within and 27% around CAA grade 1 vessels (n=60); (20% within and 0% around control vessels (n=60))	Controls that died without CAA and ICH	6	79	50	3	69	66			

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessment method	Reason authors chose this marker	BBB dysfunction marker	Location	Assessment method	Assessment method	Mean(s) marker of BBB dysfunction study sample	Mean(s) marker of BBB dysfunction control sample	Control sample	Study sample size	Study sample age	Study sample size	Study sample age	Control sample size	Control sample age
Zhao et al., 2015 ³⁶	Pathologic evaluation/microscope (n/a)	H&E, Perls' blue	MMPs have been associated with BBB dysfunction	% of vessel immunopositive for MMP-9	n/a	immunohistochemistry	immunohistochemistry	79%+/- 23.9% of CAA affected vessels	5%+/- 1.3% of control vessels	Non-demented controls	10	77	10	77	5	76
Duits et al., 2015 ³⁷	MRI (100%)	T2*-weighted images	MMPs have been associated with BBB dysfunction	CSF levels of gelatinases and their tissue inhibitors	100% lobar	Beads-based immunoassay	Beads-based immunoassay	n/a	n/a	AD/VaD without CMBs, cognitively normal controls	34	n/a	34	n/a	27 + 26	n/a
Chenget al., 2014 ³⁸	Microscope (n/a)	Perls' blue	Reduction of TJ proteins reflects BBB dysfunction	Occludin and ZO-1 expression in tissue and leptomenin geal vessels	n/a	Immunohistochemistry /western blot	Immunohistochemistry	n/a	n/a	Controls that died without CAA	9	89	9	89	10	87

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessment method hemorrhage	Location hemorrhage	BBB dysfunction marker	Reason authors chose this marker	Assessment method BBB dysfunction	Mean(sd) marker of BBB dysfunction study sample	Mean(sd) marker of BBB dysfunction control sample	Contr ol sample	Study sample size	age	% mal e	Study sample size	age	% mal e	Control sample size	age	% mal e
Hartz et al., 2012 ³⁹	MRI (100%)	T2*-weighted images	68% lobar ICH, 89% lobar CMB	% with CE lesions	Contrast agent extravasates when BBB dysfunction al	Post-contrast T1-weighted images	2/19 (11%) cases	17/19 (89%) cases	Absent	19	75	53	n/a	n/a	53	n/a	n/a	n/a

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessment method hemorrhage	Location hemorrhage	BBB dysfunction marker	Reason author chose this marker	Assessment method BBB dysfunction	Mean(sd) marker of BBB dysfunction study sample	Mean(sd) marker of BBB dysfunction control sample	Contr ol sample	Study sample size	Study sample age	Study sample % male	Control sample size	Control sample age	Control sample % male
Van Assem et al., 2012 ⁴⁰	MRI (100%)	SWI	100% lobar	Binding potential of radiolabelled Pgp substrate (R)-[¹¹ C]verapamil		PET	2.20+/- 0.34 global; 2.15+/- 0.42 frontal; 2.16+/- 0.33 parietal; 2.31+/- 0.35 temporal; 2.23+/- 0.27 occipital; 2.17+/- 0.44 posterior cingulate; 1.95+/- 0.42 anterior cingulate; 3.12+/- 0.52 medial temporal lobe; 1.94+/- 0.28 cerebellum	2.15+/- 0.34 global; 2.10+/- 0.33 frontal; 2.15+/- 0.36 parietal; 2.23+/- 0.39 temporal; 2.20+/- 0.33 occipital; 2.12+/- 0.44 posterior cingulate; 2.14+/- 0.36 anterior cingulate; 2.82+/- 0.48 medial temporal lobe; 2.03+/- 0.27 cerebellum	AD without CMBs	6	66	100	12	63	58

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessment method	Location	BBB dysfunction marker	Reason authors chose this marker	Assessment method	Mean(sd) marker of BBB dysfunction on study sample	Mean(sd) marker of BBB dysfunction on control sample	Contrast	Study sample size	Study sample age	Study sample % male	Control sample size	Control sample age	Control sample % male
Aksoy et al., 2013 ⁴¹	MRI (100%)	T2*-weighted images	48% lobar, 28% deep, 16% infratentorial	Median k-trans value within ROI contralateral to ICH (gadolinium leakage rate)	Contrast agent extravasates when BBB dysfunctional	DCE-MRI	12/25(48%)	n/a	Absent	25	65	36	n/a	n/a	n/a
Kidwell et al., 2011 ⁴²	MRI (100%)	T2*-weighted images	n/a	Sulcal or ventricular CE	Contrast agent extravasates when BBB dysfunctional	FLAIR images	39/46(85%)	n/a	Absent	46	65	40	n/a	n/a	n/a
McCourt et al., 2015 ⁴³	CT (100%)	Non-contrast CT	74% deep, 24% lobar, 2% infratentorial	Permeability surface area product in ipsilateral vs contralateral hemisphere	Contrast agent extravasates when BBB dysfunctional	Post-contrast CT	4.2+/-2.1 mL/100mL per minute	3.7+/-1.6mL/100mL per minute	Absent	53	71	72	n/a	n/a	n/a

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessment method hemorrhage	Location hemorrhage	BBB dysfunction marker	Reason authors chose this marker	Assessment method BBB dysfunction	Mean(sd) marker of BBB dysfunction study sample	Mean(sd) marker of BBB dysfunction control sample	Control	Study sample size	age	% male	size	age	% male	Control sample
Tran et al., 2016 ⁴⁴	ICH symptoms /microscopic (100%)	H&E	n/a	Relative fluorescence units per gram tissue	Reduction of TJ proteins reflects BBB dysfunction	Fluorescence intensity analysis	n/a	n/a	Controls that died without ICH	9	57	66	10	52	80	
Shams et al., 2016 ⁴⁵	MRI (100%)	T2*-weighted imaging and SWI	n/a	CSF/serum albumin ratio	Elevated CSF/serum albumin ratio reflects BBB dysfunction	ELISA	6.7	6.1	Memory clinic patient without CMBs	214 (118 multiple CMBs)	n/a	n/a	82	n/a	n/a	n/a
Poliakov et al., 2016 ⁴⁷	MRI (100%)	SWI	n/a	CSF/serum albumin ratio	Elevated CSF/serum albumin ratio reflects BBB dysfunction	Latex aggregation method and Brom-Cresol Green method	8.3+/-1.9	5.4+/-2.9	Patients with cognitive decline without CMBs	15	n/a	n/a	13	n/a	n/a	n/a

Study	Hemorrhage marker (% study sample with hemorrhage)	Assessment method	Location	BBB dysfunction marker	Reason authors chose this marker	Assessment method	Mean (sd) marker of BBB dysfunction on study sample	Mean (sd) marker of BBB dysfunction on control sample	Contrast	Study sample size	Study sample age	Study sample % male	Control sample size	Control sample age	Control sample % male
Zhang et al., 2016 ⁴⁶	MRI (100%)	SWI	62% strictly lobar,	serum VEGF levels	VEGF plays an important role in the control of vascular permeability	ELISA	336.72+/-15.18 pg/mL	192.37+/-11.34pg/mL	AD without CMBs	47	77	45	99	70	42
Goos et al., 2012 ⁴⁸	MRI (100%)	T2*-weighted images	65% strictly lobar	CSF/serum albumin ratio	Elevated CSF/serum albumin ratio reflects BBB dysfunction	nephelometry	6.7+/-3	7.4+/-4	AD without CMBs	26	67	62	26	67	62

Abbreviation key: BBB = blood-brain barrier; sd = standard deviation; DCE-MRI = dynamic contrast enhanced magnetic resonance imaging; CE = contrast enhancement; ICH = intracerebral hemorrhage; ECs = endothelial cells; FLAIR = fluid-attenuated inversion recovery; ZO-1 = zona occludens 1; VaD = vascular dementia; AD = Alzheimer's disease; CAA = cerebral amyloid angiopathy; VEGF = vascular endothelial growth factor; ELISA = enzyme-linked immunosorbent assay; CSF = cerebrospinal fluid; pgp = p-glycoprotein; CT = computed tomography; H&E = hematoxylin and eosin; MMP = matrix metalloproteinase; CMB = cerebral microbleed; SP-SHR = stroke-prone spontaneously hypertensive rats; SWI = susceptibility weighted imaging; IgG = immunoglobulin G; HRP = horseradish peroxidase; ROI = region of interest

CHAPTER 6

BLOOD-BRAIN BARRIER LEAKAGE AND MICROVASCULAR LESIONS IN CEREBRAL AMYLOID ANGIOPATHY: A *POST* *MORTEM* MRI-HISTOPATHOLOGY STUDY.

Whitney M. Freeze, Brian J. Bacskai, Matthew P. Frosch, Heidi I.L. Jacobs, Walter H. Backes,
Steven M. Greenberg, and Susanne J. van Veluw

ABSTRACT

Introduction: Cerebral amyloid angiopathy (CAA) is associated with microvascular brain injury and cognitive decline. The pathophysiology of CAA and its associated brain injury remains poorly understood. In this *post mortem* study, we explored whether blood-brain barrier (BBB) leakage is associated with CAA severity and microvascular lesions.

Methods: Intact hemispheres from six CAA cases (mean $\pm$ SD age=71.8 $\pm$ 7.1 years, five males) and two control cases without neurologic disease (aged 90 and 95 years, one male) were subjected to *post mortem* 3 tesla MRI, and cerebral microbleeds (CMB) and cortical microinfarcts (CMI) were counted. Cortical sections were sampled from each lobe, and CAA severity and BBB leakage in the form of vascular deposition and extravasation of common plasma proteins (immunoglobulin G [IgG] and fibrin) were assessed with immunohistochemistry. We examined whether BBB leakage markers were increased in CAA cases compared to controls, and whether leakage markers were increased in parieto-occipital brain regions compared to fronto-temporal brain regions within cases. We also examined whether BBB leakage was associated with CAA severity and number of microvascular lesions on MRI, and whether BBB leakage was evident in the vicinity of pathologically confirmed microhemorrhages and CMI.

Results: The percent area positive for cortical plasma protein extravasation was elevated in parieto-occipital brain regions in CAA cases (fibrin median=3.0, IQR=1.8-6.1; IgG median=1.5, IQR=0.9-3.3) compared with controls (fibrin percent 0.4 and 2.3; IgG percent 0.4 and 0.8). Within CAA cases, Wilcoxon signed-rank tests showed increased cortical fibrin and IgG extravasation in parieto-occipital brain regions compared to fronto-temporal brain regions (both comparisons $p=0.03$). Moreover, the percentage of fibrin and IgG positive vessels was positively correlated with vascular amyloid deposition within the occipital lobe (Spearman's rho fibrin=0.89, $p=0.02$; Spearman's rho IgG=0.83, $p=0.04$). Vascular IgG deposition was associated with the number of CMB rated on *post mortem* MRI (Spearman's rho=0.94, $p=0.005$). Finally, we observed fibrin deposition in walls of vessels involved in microhemorrhage, and cellular uptake of fibrin surrounding microhemorrhages and CMI.

Discussion: BBB leakage in the form of cortical deposition of plasma proteins is associated with CAA severity, and number of CMB on *post mortem* MRI. Future studies in larger samples are needed to confirm these exploratory findings.

BACKGROUND

Cerebral amyloid angiopathy (CAA) is characterized by accumulation of amyloid- β (A β) within the walls of cortical and leptomeningeal blood vessels. This type of cerebral small vessel disease is common in the aging population and found in approximately 33% of general autopsies and up to 90% of individuals with Alzheimer disease (AD) ^{1,2}. CAA is well recognized as the most common cause of lobar intracerebral hemorrhage (ICH) ³ and is believed to play a fundamental role in the development of microvascular lesions, including cerebral microhemorrhages and cerebral microinfarcts (CMI) ^{4,5}. CAA is also associated with other, more global types of brain injury, including cerebral atrophy, white matter damage, and structural network disruption ^{6,7}. Importantly, there is growing evidence that CAA has a substantial impact on age-related cognitive decline, even in the absence of lobar ICH and independent of the severity of classical AD pathology (i.e. A β plaques and neurofibrillary tangles) ^{8,9}. This vascular cognitive impairment may result from both numerous microvascular lesions as well as global atrophy and white matter damage, though the pathophysiology underlying CAA-related brain injuries is not well understood.

Previous studies have suggested a possible role of blood-brain barrier (BBB) disruption in the etiology of CAA ^{6,10,11}. The BBB is a unique feature of the cerebral microvasculature that is formed by an interactive cellular complex that involves a line-up of endothelial cells held together by tight junctions and supported by surrounding mural cells and glial cells ¹². Together, these cells selectively regulate molecular exchange between the blood and cerebral tissue. CAA-positive vessels exhibit several changes, including loss of smooth muscle cells, reduced cerebral blood flow, and vessel wall thickening ^{3,13,14}. These morphological changes to the microvasculature likely affect the integrity of the BBB ^{15,16}. Loss of BBB integrity has been suggested as a general mechanism for small vessel disease-related brain tissue injury and vascular cognitive impairment ¹⁷.

The aim of this exploratory study was to examine BBB leakage in cases with definite CAA, by quantifying the degree of vascular deposition and extravasation of plasma proteins fibrin and immunoglobulin G (IgG). We hypothesized that BBB leakage would be increased in parieto-occipital brain regions compared with fronto-temporal brain regions within CAA cases, because CAA preferentially affects the posterior lobes ^{18,19}. We also assessed the number of microvascular lesions on *post mortem* magnetic resonance imaging (MRI) and related these to markers of BBB leakage as assessed with histopathology. Finally, we explored whether markers of BBB leakage were evident in the vicinity of pathologically confirmed microhemorrhages and CMI. Two non-neurological control cases were added as a comparison. The overall goal of this study was to improve our understanding of the occurrence and impact of BBB leakage in CAA as a novel potential target for treatment.

METHODS

Human brain tissue

Intact human brain hemispheres from six CAA cases and two non-neurological control cases were received through an ongoing *post mortem* brain MRI study at Massachusetts General Hospital (MGH). If one or more large ICH(s) were present (N=5), the least affected hemisphere was selected. Otherwise, one hemisphere was randomly picked. All CAA cases were patients who were diagnosed with possible or probable CAA during life ²⁰ and who donated their brain to the J. Philip Kistler Stroke Research group or who came to autopsy through the MGH neuropathology service. The control cases were received through the MGH neuropathology service. Each patients' diagnosis was confirmed through medical records and available clinical MRI or computed tomography obtained during life, and validated upon autopsy. Control cases had no clinical records of neurological conditions or brain lesions during life, which was confirmed at autopsy. Informed consent was obtained from a legal representative prior to autopsy for all cases.

After autopsy, the hemispheres were fixed in 10% formalin for at least three weeks. Prior to *post mortem* MRI, hemispheres were packed in a plastic bag, filled with periodate-lysine-paraformaldehyde fixative, and vacuum sealed. The packed hemispheres were held at 4°C until the day before the MRI, when they were kept at room temperature. Any remaining air bubbles were removed, followed by re-sealing of the bag.

The use of the brain specimens was in accordance with the rules and regulations of the MGH institutional review board.

Post mortem MRI acquisition and analysis

Each hemisphere was subjected to *post mortem* MRI for the identification of cerebral microbleeds (CMB) (the radiological equivalent of cerebral microhemorrhages) ²¹ and cortical CMI. Hemispheres were scanned overnight for approximately 14 hours on a 3 Tesla MR system (Siemens Magnetom TrioTim syngo) using a 32-channel head coil. The scan protocol included the following sequences relevant to this study: 1) T2-weighted turbo-spin echo (TSE) (voxel size 500x500x500 μm^3 ; echo time (TE) 61 ms; repetition time (TR) 1800 ms; flip angle (FA) 150°; total scan time 3 hours 8 min 11 sec), and 2) gradient-echo (GRE) fast low angle shot (FLASH) (voxel size 500x500x500 μm^3 ; TE₁ 4.49 ms; TE₂ 11.02 ms; TR 20 ms; FA 10°, 20°, 30°; 2 averages; total scan time 1 hour 59 min). Microvascular lesions within the cortical grey matter were identified by an experienced rater (S.J.v.V.) who was blinded to CAA severity or other histopathologic findings. CMI were defined as hyperintense cortical foci on T2-weighted images and isointense on GRE images as previously described ²². CMB were counted on GRE images, appearing as homogeneous

round or ovoid foci of low signal intensity^{23, 24}. Because the *post mortem* GRE sequence is susceptible to artifacts caused by remaining air bubbles between gyri, the T2-weighted scan was also visually inspected to discriminate actual CMB from artifacts caused by air bubbles.

Tissue sampling

For each case, four cortical tissue blocks were sampled (from the frontal, temporal, parietal, and occipital lobe). In addition, in one case with the highest lesion burden, an area with multiple CMB on *post mortem* MRI was sampled for histopathological examination of the vessels involved in microhemorrhages (case #2, table 1).

Histopathological analysis

Systematically selected samples were dehydrated, embedded in paraffin, and cut into 6- μ m serial sections on a microtome. The first two sections of each block were stained with standard Hematoxylin and Eosin (H&E; to identify cortical microvascular lesions) and Luxol fast blue (to identify the white matter). Adjacent sections underwent immunohistochemistry against A β , fibrin, and IgG. Sections were deparaffinized and rehydrated through xylene and graded series of ethanol (100%, 95%, 70%) and water. Endogenous peroxidase activity was quenched by incubating the sections in 3% hydrogen peroxide solution for 20 minutes. Antigen retrieval was performed by incubating the sections in formic acid for 5 minutes (A β -staining) or with heat induced epitope retrieval in citrate buffer for 20 minutes (fibrin- and IgG-staining). Sections were blocked for one hour with normal horse or goat serum diluted in tris buffered saline (TBS). Next, sections were incubated overnight at 4° Celsius with primary antibodies against human A β (mouse monoclonal A β (clone 6F/3D) 1:200, Dako, Denmark), human fibrin (rabbit polyclonal fibrin, 1:500, Dako, Denmark), and human IgG (rabbit polyclonal IgG, 1:500, Dako, Denmark) diluted in TBS. The next day a standard avidin-biotin complex (ABC) method (Vectastain ABC kit from Vector Laboratories, Inc.) was applied and the signal was visualized using the chromogen 3,3'-Diaminobenzidine (DAB). Between all incubation steps, sections were washed in TBS. Sections were counterstained with hematoxylin for 10 seconds, dehydrated through a series of ethanol (70%, 95%, 100%) and xylene, and cover slipped using Permount™ mounting medium. Negative controls were established by omitting the primary antibody. The fibrin and IgG stainings were performed within one single batch, to avoid batch effects, and DAB developing time was held constant. The additional CMB-rich sample underwent serial sectioning as a whole. Sections ~200 μ m apart were stained with H&E, and the adjacent sections with A β and fibrin respectively.

Vascular and parenchymal A β quantification

CAA severity was evaluated on A β -stained sections by a single experienced rater (S.J.v.V.). Cortical CAA was scored using a 4-point scale; absent (0), scant A β deposition (1), some circumferential A β (2), widespread circumferential A β (3) ²⁵. Scores from the four cortical areas were added to form a single cumulative cortical CAA severity score (0-12). Parenchymal A β plaques were scored as absent (0) or present (1). Scores from each cortical area were added to form a single cortical A β plaque score (0-4).

Microscopic analyses

Sections were imaged with brightfield using the Hamamatsu NanoZoomer Digital Pathology (NDP)-HT scanner (C9600-12, Hamamatsu Photonics K.K., Japan), and a 20x objective. The viewing platform NDP.View (version 2.6.13) was used to analyze the digital sections. For the analysis of vascular protein deposition, three rectangular regions of interest (ROIs) of 8.95 mm² were manually placed at random locations within the cortex on each digital section (A β , fibrin, and IgG stained sections) (figure S1). Caution was taken to omit damaged areas on the sections and the ROIs were placed while blinded to pathology burden at low magnification (0.16x). CMI or microhemorrhages, if present, were not included in ROIs. ROI placement was guided by the section stained for myelin to assure correct positioning within cortical tissue. Within each ROI, the number of vessels that were positive and negative for CAA, fibrin or IgG was counted by a single rater (W.M.F.). Cortical vessels ≤ 20 μ m and all leptomeningeal vessels were excluded. Vessels were regarded as positive when clear immunoreactivity against A β , fibrin or IgG was evident within the vessel wall, but not when immunoreactivity was only apparent within the lumen. Within each of the three ROIs the percentage of positive vessels was calculated and averaged to obtain one value per section. A second rater (S.J.v.V.) independently rated the number of positive and negative vessels on 27 randomly selected ROIs. Both raters were blinded to clinical and MRI information and brain region.

Plasma protein extravasation was determined using digital image analysis with ImageJ free software (FIJI version 2.0.0) ²⁶. The percent area fraction of fibrin and IgG labeling of cortical gray matter was determined using image thresholding (Methods and figure S2 in the online data supplement).

CMIs were evaluated by a single experienced rater (S.J.v.V.) on the H&E-stained sections, defined as areas of tissue pallor, with evidence of cell loss and gliosis. Microhemorrhages were evaluated on the serial H&E-stained sections obtained from the additional CMB-rich sample, and

identified by evidence of erythrocyte extravasation (i.e. acute microhemorrhage) or blood-breakdown products including hematoidin or hemosiderin (i.e. subacute microhemorrhage) ²¹.

Statistical analysis

A threshold of $\alpha < .05$ was used to determine statistical significance. Inter-rater reliability was determined using the intra-class correlation coefficient (icc) based on a single rating, absolute agreement two-way random effects model ²⁷. We did not statistically assess differences on the BBB leakage measurements between CAA cases (n=6) and controls (n=2) because the power of the non-parametric Mann-Whitney U test was too low to reach the $\alpha < .05$ level, and we considered parametric testing not appropriate given the small sample size ²⁸. We therefore report the median values for the leakage measures for CAA cases and controls. Wilcoxon-signed rank tests were applied to assess differences in BBB leakage measures in fronto-temporal (average of frontal and temporal cortex) vs parieto-occipital (average of parietal and occipital cortex) brain regions within the CAA cases (n=6). Associations between continuous variables were determined with Spearman's rho correlation coefficient. All P-values are two-tailed and we did not correct for multiple comparisons because this was an exploratory study. All analyses were conducted with R statistical software (R version 3.3.3) ²⁹.

RESULTS

Characteristics for each case are listed in Table 1. The mean $\pm$ SD age of the six CAA patients was 71.8 $\pm$ 7.1 years, and five were male. The two control participants were 90 and 95 years old, and one was male. Inter-rater reliability for the assessment of the number of positive vessels (n=27 regions) was good to excellent (icc=0.894, 95% confidence interval (CI)=0.77-0.95), and for the assessment of the number of negative vessels poor to moderate (icc=0.248, 95% CI=-0.10-0.60).

Table 1: Case characteristics

Case #	Age at death (y)	Sex	PMI	Diagnosis	Cortical CAA score	A β plaque score	MRI CMB count	Histo CMH count	MRI CMI count	Histo CMI count
1	80	M	Unk	CAA	5	3	41	0	115	17
2	70	M	16h	CAA	9	3	261	1	33	2
3	65	M	27h	CAA	7	3	39	0	21	10
5	65	M	14h	CAA	7	1	85	3	144	3
7	81	M	Unk	CAA	5	4	4	0	12	3
8	70	F	Unk	CAA	6	4	13	0	7	1
4	90	M	6h	Control	0	4	0	0	3	1
6	95	F	4h	Control	0	1	1	0	1	0

Abbreviations: y, years; PMI, *post mortem* interval; Unk, unknown; CAA, cerebral amyloid angiopathy; MRI, magnetic resonance imaging; Histo, histopathology; CMB, cerebral microbleeds; CMH, cerebral microhemorrhages; CMI, cerebral microinfarcts.

Plasma protein extravasation between and within cases

The median cortical fraction positive for plasma proteins fibrin and IgG appeared higher in parieto-occipital brain regions but not in fronto-temporal regions, in CAA cases (fibrin parieto-occipital regions median=3.0, IQR=1.8-6.1; IgG parieto-occipital regions median=1.5, IQR=0.9-3.3) compared to controls (fibrin percent parieto-occipital regions 0.4 and 2.3; IgG percent parieto-occipital regions 0.4 and 0.8) (Figure 1). Within the CAA cases, the cortical fraction positive for fibrin and IgG was significantly higher in parieto-occipital brain regions compared with fronto-temporal brain regions (Figure 1; both comparisons $W=0$, $p=0.03$).

Vascular deposition of plasma proteins between and within cases

The median percentage of positive vessels for fibrin and IgG also appeared higher in patients compared to controls in parieto-occipital brain regions (figure S3). Within the CAA cases, there was a trend towards a higher percentage of vessels positive for IgG in parieto-occipital brain regions compared with fronto-temporal brain regions ($W=1$, $p=0.06$), but not for fibrin ($W=5$, $p=0.3$) (figure S3).

Figure 1: Percentage of cortical area that is positive for fibrin or IgG

Associations between A) cortical fraction positive for fibrin within CAA cases and controls and B) cortical fraction positive for IgG within CAA cases and controls. Mann-Whitney U tests could not be computed at the $\alpha < .05$ level to test for differences between CAA cases ($n=6$) and controls ($n=2$). Within CAA cases, Wilcoxon-signed rank tests showed significant differences between fronto-temporal and parieto-occipital brain regions in cortical fibrin and IgG fractions, indicated as $p < 0.05$.

Associations between CAA severity score and vascular deposition of A β , fibrin, and IgG

Whole brain cortical CAA score was significantly associated with the percentage of vessels positive for plasma proteins averaged across lobes (Spearman's $\rho=0.88$, $p=0.02$ for both comparisons) (figure 2), but not with plasma protein extravasation averaged across lobes (Fibrin: Spearman's $\rho=-0.09$, $p=0.87$; IgG: Spearman's $\rho=-0.53$, $p=0.28$).

Local CAA severity in the form of percentage of vessels positive for A β was significantly associated with the percentage of vessels positive for fibrin (Spearman's $\rho=0.89$, $p=0.02$) and IgG (Spearman's $\rho=0.83$, $p=0.04$) within the occipital lobe (figure S4).

Associations between CAA, vascular deposition of fibrin and IgG, and CMBs

The number of CMB counted on MRI was significantly positively associated with the percentage of IgG positive vessels averaged across lobes (Spearman's $\rho=0.94$, $p=0.005$), but not with the percentage of fibrin positive vessels (Spearman's $\rho=0.54$, $p=0.27$), or the percentage of A β positive vessels (Spearman's $\rho=0.60$, $p=0.21$) (figure 3).

Figure 2: Cerebrovascular deposition of amyloid- β and plasma proteins, and associations between whole brain CAA severity and vessels positive for plasma proteins

Immunohistochemistry against amyloid- β (A β), fibrin, and immunoglobulin G (IgG) was performed on cortical brain sections, and the percentage of positive vessels was determined. Cortical CAA severity within each case was scored using a continuous rating scale. A) representative images of A β , fibrin, and IgG staining in CAA case #2. B) Data points representing the cortical CAA severity score against whole brain percentage of cortical vessels positive for fibrin and IgG. Only CAA cases ($n=6$) were included in the analyses.

Figure 3: Number of CMB and percent vessels positive for A β and plasma proteins

Data points representing the number of cerebral microbleeds rated on *post mortem* MRI against the percentage of positive vessels averaged across lobes for A) Amyloid- β , B) Fibrin, and C) IgG. Spearman's rank correlations with corresponding p-values are depicted in each graph. Only CAA cases were included in the analyses.

Associations between CAA, vascular deposition of fibrin and IgG, and CMIs

The number of CMI counted on MRI was not significantly associated with the percentage of A β , fibrin, or IgG positive vessels averaged across lobes (A β : Spearman's $\rho=0.31$, $p=0.54$; Fibrin: Spearman's $\rho=0.20$, $p=0.70$; IgG: Spearman's $\rho=0.66$, $p=0.16$).

Fibrin in the vicinity of cerebral microhemorrhage and CMI

Histopathological examination of cerebral microhemorrhages revealed fibrin deposition within the walls of the involved vessel (figure 4), and fibrin uptake in neurons and astrocytes surrounding the hemorrhagic lesions. Cellular uptake of fibrin and IgG was also frequently observed in the vicinity of CMIs (figure S5), but this was not a finding specific for microhemorrhages or CMI.

Figure 4: BBB leakage surrounding a microhemorrhage

A cortical microbleed observed on *post mortem* MRI in case #2 was sampled for histopathological analysis (A, hematoxylin and eosin stain). Fibrin deposition (C), but not A β accumulation (B), was present in the wall of the vessel that was involved in the microhemorrhage. On the magnified image, immunopositivity for fibrin can be observed in astrocytes (D). Scale bar in A, B and C is 500 μm , D= 250 μm .

DISCUSSION

The aim of this exploratory *post mortem* study was to assess associations between markers of BBB disruption, CAA severity, and microvascular lesions to evaluate whether BBB disruption might play a possible role in CAA-related brain injury. Our data shows increased extravasation and vascular deposition of plasma protein in parieto-occipital brain regions compared with fronto-temporal brain regions within CAA cases, which was not evident in older non-neurological control cases. We also found that cerebrovascular deposition of plasma proteins was positively associated

with CAA severity, and with total number of CMBs detected on MRI. Histological examination of microvascular lesions showed fibrin deposition within the wall of vessels presumably involved in microhemorrhage, and cellular uptake of fibrin surrounding both microhemorrhages and CMI.

Plasma protein extravasation and cellular uptake of plasma proteins have been previously demonstrated in the cerebral deep gray matter, white matter, and cortex in normal aging, cerebral small vessel disease and AD³⁰⁻³². Our observation of increased plasma protein extravasation and vascular deposition of plasma proteins in parieto-occipital brain regions within CAA cases is in line with the predilection of CAA pathology and associated lesions for parieto-occipital brain regions^{18, 19}. The observed increase in BBB disruption in parieto-occipital brain regions in CAA cases compared with controls is also interesting considering that BBB leakage is a common feature of aging and the controls were much older (aged 90 and 95 years) than the CAA cases (mean age 72 years)³³. The observed immunolabeling of fibrin within cells surrounding microvascular lesions suggests that leaked plasma proteins can be absorbed by local neurons and glial cells, which could be a contributing factor of widespread neuronal network damage in CAA³⁴.

Our observation of increased frequency of vascular deposition of plasma proteins with increasing CAA severity is in accordance with previous studies showing more frequent plasma protein deposition within CAA-positive vessels compared with normal vessels^{15, 35}. Interestingly, when we examined the association between vascular A β deposition and vascular plasma protein deposition, this association was most prominent within the occipital lobe, where CAA severity is highest.

Although the BBB is often described as a feature of the capillary vessels, we and others^{15, 35} examined vascular deposition of fibrin and IgG within relatively large vessels ($\geq 20\ \mu\text{m}$). Deposition of plasma proteins within these small arteries, arterioles and/or veins could manifest through structural vessel wall damage in the form of weakened tight junctions between endothelial cells. An alternative explanation may be that leakage of plasma proteins occurs at the capillary level, and that extravasated proteins are transported towards larger vessels via perivascular clearance pathways. Failure of perivascular clearance could result in deposition of these proteins into the vessel wall, similarly to what has been suggested to occur with A β in CAA³⁶. Examination of plasma protein leakage at the capillary level and the role of perivascular clearance pathways would be an interesting topic for future studies, though discriminating plasma protein deposition within the vessel wall from plasma proteins within the capillary lumen might be challenging.

It has been previously suggested that microhemorrhages may represent the most severe form of BBB disruption, as the BBB also provides protection against red blood cell extravasation³⁷. A recent clinical case study supported this idea by revealing BBB leakage in the form of local extravasation of a gadolinium-based contrast agent prior to CMB formation on MRI¹¹. Our results are in line with these findings by showing positive associations between BBB leakage of IgG and overall CMB quantity, and vascular deposition of fibrin in vessels involved in microhemorrhage. However, it remains unclear whether there is a causal relationship between BBB disruption and microhemorrhages based on the present results. Signs of BBB leakage surrounding a (micro)hemorrhage could also be a secondary consequence of vessel rupture³⁸.

Studies examining the relationship between CMI and BBB leakage in CAA are scarce. Fibrin is an interesting protein in the context of CMI and CMB in CAA because of its essential role in coagulation. Previous work has shown that interactions between fibrin and A β may lead to the formation of abnormal blood clots with resistance to lysis³⁹. It could be hypothesized that such abnormal clots form a source for CMI formation in CAA. However, whether BBB leakage of fibrin precedes or follows CMI formation needs further exploration.

Strengths of this study are the use of *post mortem* MRI scans of intact hemispheres of well-characterized cases to assess microvascular lesions throughout the brain. Unfortunately, only a small number of control cases were available for *post mortem* scanning, which limited our statistical power for between-group (i.e. CAA vs controls) comparisons. Despite this limitation, sufficient variation in CAA severity and microvascular lesions enabled us to detect significant associations with local BBB leakage within CAA cases. Because this was an exploratory study we did not correct for multiple comparison in our statistical analyses, which may have potentially resulted in false positive findings. Because microhemorrhages often escape detection on routine histopathological examination (in contrast to CMI), we added additional serial sections from the case with the most CMBs on MRI. These case-specific microhemorrhages might not have been representative for all cases. Another limitation is the fact that for three cases the *post mortem* interval was unknown, and *post mortem* interval was shortest for the two control cases, which may have resulted in a source of bias. However, previous studies in larger samples show no significant associations between vascular deposition or extravasation of plasma proteins and *post mortem* interval^{15, 30}. The inter-rater variability of negative vessels was poor to moderate, which might also form a potential source of bias. All findings will require replication in independent study samples. Further studies in larger

human samples compared with longitudinal preclinical studies are needed to better address the question as to whether BBB disruption plays a fundamental role in CAA-related brain injury.

CONCLUSION

In this exploratory study we observed associations between BBB leakage of plasma proteins and CAA severity assessed on histological sections, and these measures were related to the number of CMBs rated on *post mortem* MRI. BBB leakage of plasma proteins was evident surrounding pathologically confirmed microhemorrhages and CMI. Our observations suggest that BBB disruption might play a role in the pathogenesis of CAA-related brain injury, and thus represent a target for future interventions aimed at preventing cognitive impairment in CAA.

REFERENCES

1. Jellinger KA, Attems J. Prevalence and pathogenic role of cerebrovascular lesions in Alzheimer disease. *J Neurol Sci* 2005;229-230:37-41.
2. Maia LF, Mackenzie IR, Feldman HH. Clinical phenotypes of Cerebral Amyloid Angiopathy. *J Neurol Sci* 2007;257:23-30.
3. Vinters HV. Cerebral amyloid angiopathy. A critical review. *Stroke* 1987;18:311-324.
4. Soontornniyomkij V, Lynch MD, Mermash S, et al. Cerebral microinfarcts associated with severe cerebral beta-amyloid angiopathy. *Brain pathology (Zurich, Switzerland)* 2010;20:459-467.
5. Olichney JM, Ellis RJ, Katzman R, Sabbagh MN, Hansen L. Types of cerebrovascular lesions associated with severe cerebral amyloid angiopathy in Alzheimer's disease. *Ann N Y Acad Sci* 1997;826:493-497.
6. Charidimou A, Boulouis G, Gurol ME, et al. Emerging concepts in sporadic cerebral amyloid angiopathy. *Brain : a journal of neurology* 2017;140:1829-1850.
7. Smith EE. Cerebral amyloid angiopathy as a cause of neurodegeneration. *J Neurochem* 2017.
8. Greenberg SM, Gurol ME, Rosand J, Smith EE. Amyloid angiopathy-related vascular cognitive impairment. *Stroke* 2004;35:2616-2619.
9. Arvanitakis Z, Leurgans SE, Wang Z, Wilson RS, Bennett DA, Schneider JA. Cerebral amyloid angiopathy pathology and cognitive domains in older persons. *Annals of neurology* 2011;69:320-327.
10. Fisher M, French S, Ji P, Kim RC. Cerebral microbleeds in the elderly: A pathological analysis. *Stroke* 2010;41 (4):e377.
11. van Nieuwenhuizen KM, Hendrikse J, Klijn CJM. New microbleed after blood-brain barrier leakage in intracerebral haemorrhage. *BMJ Case Rep* 2017;2017.
12. Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV. Establishment and Dysfunction of the Blood-Brain Barrier. *Cell* 2015;163:1064-1078.
13. Greenberg SM, Vonsattel JP. Diagnosis of cerebral amyloid angiopathy. Sensitivity and specificity of cortical biopsy. *Stroke* 1997;28:1418-1422.
14. Vonsattel JP, Myers RH, Hedley-Whyte ET, Ropper AH, Bird ED, Richardson EP, Jr. Cerebral amyloid angiopathy without and with cerebral hemorrhages: a comparative histological study. *Annals of neurology* 1991;30:637-649.
15. Hultman K, Strickland S, Norris EH. The APOE varepsilon4/varepsilon4 genotype potentiates vascular fibrin(ogen) deposition in amyloid-laden vessels in the brains of Alzheimer's disease patients. *J Cereb Blood Flow Metab* 2013;33:1251-1258.
16. Hernandez-Guillamon M, Martinez-Saez E, Delgado P, et al. MMP-2/MMP-9 plasma level and brain expression in cerebral amyloid angiopathy-associated hemorrhagic stroke. *Brain Pathology* 2012;22:133-141.
17. Wardlaw JM, Sandercock PA, Dennis MS, Starr J. Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? *Stroke* 2003;34:806-812.
18. Rosand J, Muzikansky A, Kumar A, et al. Spatial clustering of hemorrhages in probable cerebral amyloid angiopathy. *Annals of neurology* 2005;58:459-462.
19. Vinters HV, Gilbert JJ. Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes. *Stroke* 1983;14:924-928.
20. Greenberg SM, Charidimou A. Diagnosis of Cerebral Amyloid Angiopathy: Evolution of the Boston Criteria. *Stroke* 2018;49:491-497.
21. van Veluw SJ, Charidimou A, van der Kouwe AJ, et al. Microbleed and microinfarct detection in amyloid angiopathy: a high-resolution MRI-histopathology study. *Brain : a journal of neurology* 2016;139:3151-3162.
22. van Veluw SJ, Shih AY, Smith EE, et al. Detection, risk factors, and functional consequences of cerebral microinfarcts. *The Lancet Neurology* 2017;16:730-740.
23. Gregoire SM, Chaudhary UJ, Brown MM, et al. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. *Neurology* 2009;73:1759-1766.
24. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *The Lancet Neurology* 2013;12:822-838.

25. Love S, Chalmers K, Ince P, et al. Development, appraisal, validation and implementation of a consensus protocol for the assessment of cerebral amyloid angiopathy in post-mortem brain tissue. *American journal of neurodegenerative disease* 2014;3:19-32.
26. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nature methods* 2012;9:676-682.
27. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *Journal of chiropractic medicine* 2016;15:155-163.
28. Hinton PR. *Statistics explained* (3rd edition.). Hove, East Sussex: Routledge, 2014.
29. R Core Team. *R: A Language and Environment for Statistical Computing* 2016.
30. Bridges LR, Andoh J, Lawrence AJ, et al. Blood-brain barrier dysfunction and cerebral small vessel disease (arteriolosclerosis) in brains of older people. *J Neuropathol Exp Neurol* 2014;73:1026-1033.
31. Hainsworth AH, Minett T, Andoh J, et al. Neuropathology of White Matter Lesions, Blood-Brain Barrier Dysfunction, and Dementia. *Stroke* 2017;48:2799-2804.
32. Viggars AP, Wharton SB, Simpson JE, et al. Alterations in the blood brain barrier in ageing cerebral cortex in relationship to Alzheimer-type pathology: a study in the MRC-CFAS population neuropathology cohort. *Neurosci Lett* 2011;505:25-30.
33. Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease--systematic review and meta-analysis. *Neurobiology of aging* 2009;30:337-352.
34. Reijmer YD, Fotiadis P, Charidimou A, et al. Relationship between white matter connectivity loss and cortical thinning in cerebral amyloid angiopathy. *Human brain mapping* 2017.
35. Cortes-Canteli M, Paul J, Norris EH, et al. Fibrinogen and beta-amyloid association alters thrombosis and fibrinolysis: a possible contributing factor to Alzheimer's disease. *Neuron* 2010;66:695-709.
36. Weller RO, Subash M, Preston SD, Mazanti I, Carare RO. Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. *Brain pathology (Zurich, Switzerland)* 2008;18:253-266.
37. Fisher M. Cerebral Microbleeds and Thrombolysis: Clinical Consequences and Mechanistic Implications. *JAMA neurology* 2016;73:632-635.
38. Keep RF, Zhou N, Xiang J, Andjelkovic AV, Hua Y, Xi G. Vascular disruption and blood-brain barrier dysfunction in intracerebral hemorrhage. *Fluids and Barriers of the CNS* 2014;11.
39. Ahn HJ, Zamołodchikov D, Cortes-Canteli M, Norris EH, Glickman JF, Strickland S. Alzheimer's disease peptide beta-amyloid interacts with fibrinogen and induces its oligomerization. *Proc Natl Acad Sci U S A* 2010;107:21812-21817.

SUPPLEMENTARY MATERIAL

Figure S1: ROI positioning in NDP.viewer

Regions of interest were placed at random locations on the fibrin-stained section and on corresponding locations on adjacent sections stained for immunoglobulin G (IgG) and amyloid- β (A β). During ROI positioning the digital sections were displayed together to omit damaged areas on each section, and the luxol fast blue-stained section was used for guidance to ensure that ROI placement was within the cortical grey matter. The current magnification was used for ROI placement, to ensure blinding for pathology severity, as at this magnification it is not possible to distinguish individual vessels.

Supplemental methods

To determine the percent area fraction of fibrin and IgG labeling of cortical gray matter areas, the digital fibrin and IgG stained sections were opened in FIJI and saved as .tiff images. First, the color deconvolution tool was used to split the image into two separate 8-bit images, one based on the hematoxylin signal and one based on the DAB signal. To ensure unbiased ROI selection, the cortex was manually segmented on the hematoxylin digital image, guided by the Luxol fast blue-stained section. The cortical ROI was then copied onto the split DAB image. Pixels positive for fibrin and IgG were detected using a fixed threshold set at intensity 110. This threshold was manually determined *a priori* based on visual inspection of multiple images.

Figure S2: ROI placement and image thresholding in ImageJ

Manual ROI placement and image thresholding. The sections stained for fibrin and IgG were split into two digital images, one based on the hematoxylin signal and the other based on the DAB signal. A) Converted digital image based on hematoxylin counterstain signal with manually segmented cortical grey matter. B) The Luxol fast blue- stained section guided ROI placement within the cortical grey matter. C) Converted digital image based on DAB signal from fibrin staining. D) Converted digital image based on DAB signal with the ROI copied from image A. Red pixels were defined as positive for fibrin according to the set intensity threshold.

Figure S3: Total number of vessels that is positive for fibrin or IgG

Depicted is the percentage of A) fibrin and B) IgG positive vessels for CAA cases and controls within each cortical brain area. Mann-Whitney U tests could not be computed at the $\alpha < .05$ level to test for differences between CAA cases ($n=6$) and controls ($n=2$). Within CAA cases, Wilcoxon signed-rank tests showed a marginally significant difference between fronto-temporal and parieto-occipital brain regions in percentage of IgG positive vessels.

Figure S4: Associations between percentage positive vessels for fibrin, IgG and A β

Data points representing the association between A) percentage of cortical vessels positive for fibrin and percentage of cortical vessels positive for A β , and B) percentage of cortical vessels positive for IgG and percentage of cortical vessels positive for A β . Spearman's rank correlation coefficients are depicted in the figure by different colors corresponding to each lobe (from top to bottom: frontal cortex, temporal cortex, parietal cortex, occipital cortex). Only CAA cases (n=6) were included in the analysis.

Figure S5: BBB leakage surrounding a cerebral microinfarct

A cortical microinfarct was identified on histopathological examination of a systematically sampled brain section in case #3 (A, hematoxylin and eosin stain, microinfarct is indicated by the green pin). Fibrin deposition (C), but not vascular A β accumulation (B), was present in the vicinity of the ischemic lesion. On the magnified image, cellular immunopositivity for fibrin can be observed (D). Scale bar in A, B and C is 1000 μ m, D= 250 μ m.

CHAPTER 7

GENERAL DISCUSSION.

This thesis focuses on associations between blood-brain barrier (BBB) leakage, cerebral small vessel disease (cSVD), Alzheimer disease (AD) pathology, and cognition, with the aim of expanding current insights on microvascular contributions to the pathophysiology and symptomatology of dementia. From the previous chapters it can be inferred that the relation between (micro)vascular pathology, AD pathology, and cognition is complex, and that interactions between different pathological processes are very likely (figure 1). More specifically, we showed that cSVD severity was negatively associated with hippocampal volume in memory clinic patients with abnormal amyloid- β levels. Moreover, we identified a positive relationship between BBB leakage and cSVD severity, which formed a potential mechanism underlying delayed information processing speed in cognitively normal and cognitively impaired older individuals. We also demonstrated that the spectrum of vascular lesions associated with BBB leakage ranges from ischemic to hemorrhagic, and that BBB dysfunction in relation to cerebrovascular disease and dementia can be measured with various techniques (post-contrast T1-weighted MRI, post-contrast FLAIR MRI, histopathology, CSF analysis). This chapter will present my views on the dynamics of BBB dysfunction, AD pathology, and cSVD, in relation to cognitive impairment, along with a discussion of the results in a more general context. In addition, research considerations, clinical implications, and suggested directions for future research will be discussed.

Figure 1. Associations between markers of AD and markers of cerebrovascular damage identified in this thesis. Green arrows indicate positive associations, whereas red arrows indicate negative associations. The straight orange arrow indicates an interaction effect (CH2) and the curved orange arrow indicates a mediation effect (CH3). Abbreviations: AD = Alzheimer disease; CH = chapter; HV = hippocampal volume; WMH = white matter hyperintensities; cSVD = cerebral small vessel disease; CAA = cerebral amyloid angiopathy; HyV = hypertensive vasculopathy; ICH = intracerebral hemorrhage; CMB = cerebral microbleeds.

Cerebrovascular pathology and Alzheimer disease models

Many previous autopsy and observational studies have established that cerebrovascular pathology is frequently present in individuals with AD pathology¹⁻³. Moreover, cerebrovascular damage has been associated with cognitive decline and neurodegeneration, independent of concomitant AD pathology⁴⁻⁶. Despite the increasing recognition of cerebrovascular damage as a contributor to neurodegeneration and cognitive decline in the context of late-onset AD, there is no consensus as to if, when, and where vascular damage fits into the AD disease cascade model. Whereas classical AD research models do not incorporate vascular factors at all^{7,8}, there are others that postulate vascular dysregulation as one of the first and foremost events that may trigger the abnormal protein accumulation that characterizes AD⁹⁻¹¹. A more recent biomarker model of AD has suggested that adding vascular pathology to the model might be interesting, but systematic implementation across studies might be difficult because consensus about pathological and MRI scoring systems is currently lacking¹².

In light of the varying perspectives and limitations, further exploration of cerebrovascular damage on the one hand, and AD biomarkers amyloid- β plaques and neurofibrillary tangles on the other hand, in relation to neurodegeneration and cognitive decline is important to determine their relative contributions to dementia. Interestingly, our findings in chapter 2 demonstrated that amyloid- β status moderates the relationship between cSVD severity and hippocampal volume in non-demented memory clinic patients. These findings suggest that cerebrovascular disease can trigger neurodegenerative processes at an early disease stage when the brain is vulnerable due to co-existent AD pathology. When one type of pathology is already present, a second type of pathology may more easily trigger downstream processes compared to the presence of only a single type of pathology because the brain is already predisposed to damage. This idea is also consistent with previous work showing that individuals with similar dementia severities exhibit fewer AD pathology in the presence of cSVD¹³.

Where does blood-brain barrier leakage fit into the disease model?

This thesis focuses on BBB leakage as a marker of microvascular damage and addresses associations with MRI markers of cSVD and AD. The BBB is a unique feature of the cerebral blood vessels, and refers to the selective permeability and transport properties of the cerebrovascular endothelium¹⁴. The BBB protects the brain from toxic substances that reside within the blood, while delivering essential nutrients and oxygen products where necessary and

removing waste products, which is all essential for cerebral homeostasis¹⁵. Especially the latter function of the BBB has received considerable interest in AD research because of its possible role in abnormal cerebral amyloid- β accumulation. Animal studies suggest that 70-85% of amyloid- β is cleared from the brain across the BBB¹⁶⁻¹⁸. It has been suggested that the clearance system of the BBB might be dysfunctional in humans, since accumulation of amyloid- β in late-onset AD is not due to overproduction of the protein, but due to faulty clearance¹⁹. Evidence as to whether BBB dysfunction plays a role in AD remains conflicting. Two recent MRI studies in humans showed that the BBB does not function normally as leakage of contrast agent is increased throughout the brain²⁰, and specifically within the hippocampus²¹, in individuals with early AD and mild cognitive impairment (MCI) compared with cognitively normal older individuals. However, evidence for BBB leakage in AD from earlier imaging studies²²⁻²⁴ as well as biochemical^{25, 26} and histological studies^{27, 28} is less conclusive^{29, 30}.

The discrepancy between studies that address BBB leakage in AD may be related to two important methodological issues. Firstly, methods that are available for measuring BBB leakage are not very sensitive while BBB leakage in AD is subtle. Because BBB leakage has been shown to increase with normal aging as well³⁰, it may be difficult to find differences between cognitively normal older individuals and individuals with AD. Secondly, BBB leakage is strongly associated with cSVD^{31, 32} and other age-related neurological diseases³³, and because of the frequent co-occurrence of AD pathology and cerebrovascular pathology it is difficult to disentangle the proportion of BBB leakage that is associated with AD, and the proportion that is related to cSVD (or another disease). In chapter 3, we attempted to examine these complex pathways in cognitively normal older individuals and individuals with clinical diagnoses of MCI and AD. As expected, BBB leakage throughout the whole brain was associated with cSVD severity, and cSVD severity mediated the association between BBB leakage and cognition. In contrast, hippocampal BBB leakage was not associated with hippocampal volume or cognitive performance. Furthermore, in contrast with the earlier mentioned studies, we did not find any regional differences in BBB leakage between cognitively normal individuals and those with MCI or AD. Based on our study, the position of BBB leakage across the AD-cSVD spectrum seems to be more closely situated towards cSVD pathology. However, based on our results we cannot conclude whether or not BBB leakage is associated with AD independent of cSVD, because our study was cross-sectional, our sample size limited, and we did not have information about cerebral amyloid- β and tau levels.

The dynamics of BBB leakage in relation to ischemic and hemorrhagic brain lesions

As mentioned before, cerebrovascular lesions such as ischemic and hemorrhagic stroke are important contributors to cognitive impairment and dementia^{3, 34-36}. Both ischemic and hemorrhagic lesions have been associated with BBB leakage, mostly in the acute post-stroke phase³⁷⁻⁴⁰. However, associations between cerebrovascular lesions and BBB leakage have been little investigated in memory clinic patients, who frequently exhibit old cerebrovascular lesions. In chapter 4 we explored whether post-contrast FLAIR imaging could be applied to identify pericortical BBB leakage in individuals with cognitive impairment and normal cognition, and if so, what MRI markers of cerebrovascular damage were associated with this radiological phenomenon. Focal pericortical BBB leaks were identified in approximately 30 percent of the examined study sample. Interestingly, this type of BBB leakage was significantly associated with presence of old ischemic infarcts, which were identified on MRI remote from the leakage location. Moreover, the site of focal BBB leakage remained stable over time in three cases that were examined on a second occasion, approximately 2 years later. These findings indicate that chronic focal BBB leakage is associated with old ischemic infarct presence across the spectrum from normal cognition to AD type dementia.

Interestingly, a recent case study has linked pericortical BBB leakage to the development of hemorrhagic pathology by identifying a new cerebral microbleed (CMB) at the location of earlier post-contrast FLAIR pericortical enhancement⁴¹. Based on these and our findings it could be hypothesized that focal BBB leaks - as identified on post-contrast FLAIR images - represent sites of chronic vessel fragility that develop due to ischemic processes, and that these fragile vessel segments may be at risk for CMB development. CMBs have been previously suggested to be the most extreme form of BBB leakage⁴². The idea that chronic ischemic processes affect the BBB resulting in increased hemorrhagic risk is an intriguing possibility that links ischemic and hemorrhagic brain pathology in both hypertensive vasculopathy and cerebral amyloid angiopathy (CAA)^{43,44}. An increased occurrence of new CMB after ischemic stroke or thrombectomy for large vessel ischemic stroke identified by several studies is also in line with this idea⁴⁵⁻⁴⁷. A possible mechanism mediating the complex association between ischemia and hemorrhage is the release of excess vascular endothelial growth factor (VEGF) with angiogenesis and BBB leakage in response to ischemic injury^{48,49}. Serum VEGF levels were found to be increased in individuals with CMBs compared with individuals without CMBs⁵⁰. In our study in chapter 4 we did not find an association with pericortical leakage and CMB presence, but we did not distinguish between lobar and deep CMBs in our analyses and the percentage of participants with CMBs was low (i.e., 12%).

The association between lobar CMBs and BBB leakage needs further exploration in future studies with larger samples of individuals with cerebral hemorrhage.

Evidence for BBB leakage in the etiology of cSVD-related hemorrhagic lesions

In the two final chapters of this thesis we further explored the role of BBB leakage in the etiology of cSVD related intracerebral hemorrhage (ICH). In chapter 5, the available literature on BBB dysfunction in ICH and CMB was systematically addressed. Most of the studies included found signs of BBB disruption in patients and animal models with cerebral hemorrhage, despite heterogeneous study samples and methods used to measure BBB dysfunction. In chapter 6 we took a closer look at the association between microvascular lesions and BBB leakage in *post mortem* tissue of patients with CAA. This type of cSVD is prevalent in approximately a third of general autopsies and in up to 90 percent of those with AD ^{51, 52}. We first mapped the number of microvascular lesions by subjecting intact hemispheres of CAA patients to *post mortem* MRI. These microvascular lesions included cerebral microinfarcts (CMI) and CMB. Next, we systematically sampled brain tissue from each lobe and determined CAA severity and BBB leakage in the form of extravasation and vascular deposition of plasma proteins. Our results confirmed that BBB leakage was positively associated with CAA severity and number of CMB detected on *post mortem* MRI. As such, this study contributes further evidence to the possibility that BBB leakage plays a role in the formation of cerebral hemorrhage. However, it remains unknown whether similar processes are at play in AD patients with CAA, because our study sample was very small, and our selection of severe CAA cases is probably not representative of the CAA observed in the general AD population ⁵³.

Research considerations

Measuring BBB leakage in AD remains a challenge for several reasons. In the present section some important research considerations will be discussed. Firstly, BBB leakage does not seem specific to AD since it is also prevalent in other neurological diseases and in normal aging ^{33, 54}. Particularly the association between leakage and cSVD, which is frequently associated with AD, complicates the ability to draw firm conclusions on the role of BBB leakage in both diseases ⁵⁵. Therefore, the interpretation of studies that measure BBB leakage in cSVD and AD should strongly depend on the selected study population. This requires a careful description of study samples with concurrent measurement of clinical, neuropsychological, cerebrovascular, and AD biomarker profiles. In chapter 3 and chapter 4 we described associations between BBB leakage and MRI markers of AD and cSVD and cognitive performance in cognitively normal individuals and memory clinic

patients. The simultaneous measurement of BBB leakage and MRI markers of neurodegeneration and cSVD is a strength of these studies. However, a major drawback is the lack of amyloid- β and tau protein measurements. On a related note, the use of different animal models of cSVD and AD across studies make direct comparisons of the results between studies difficult, as we encountered in our systematic review in chapter 5. Preclinical animal studies are important for increasing our understanding of human disease physiology, but each different model exhibits unique features that may influence BBB permeability. Moreover, translation to human disease forms a major challenge.

Secondly, many different methods of measuring BBB leakage exist, each of which has their own advantages and disadvantages. Leakage can be measured as presence of endogenous plasma proteins in cerebrospinal fluid (CSF), within cerebral vessel walls, and within the parenchyma, or as leakage of exogenously administered contrast agents. Unfortunately, the sensitivity and specificity of each method remains elusive. In the case of measuring leakage within CSF, the source location of the leaked components remains unknown. Blood components could indirectly end up in the CSF via leakage through the parenchymal BBB and perivascular clearance pathways, or directly via the blood-CSF barrier. Also, when measuring local BBB leakage in the brain, it may still be difficult to compare studies that applied the same method because of major differences in data acquisition and preprocessing procedures between studies. This is demonstrated by a recent literature review that describes how a handful of studies that all applied the same popular imaging method for measuring and quantifying BBB disruption in dementia, dynamic contrast-enhanced (DCE) MRI, produced highly variable results ⁵⁶. A standardized comparison of effect sizes between patients and age-matched control samples may partly overcome this issue. Moreover, a recent study showed that the reproducibility of this technique is moderate-to-excellent for group studies, which is hopeful for longitudinal studies ⁵⁷. It would be interesting, after the signal-to-noise ratio of the DCE-MRI technique is optimized, to work towards harmonization of the DCE-MRI protocol to increase comparability of the results between different studies.

Advantages of measuring BBB leakage in the form of contrast agent extravasation with MRI include coverage of (almost) the entire brain and the relative non-invasiveness of the technique. Another advantage of MRI is that it can simultaneously be applied to map BBB leakage as well as different markers of neurodegeneration (such as global and hippocampal atrophy) and cerebrovascular damage (such as white matter hyperintensities, hemorrhages, and ischemic infarcts). Moreover, we have seen in chapter 3 and chapter 4 of this thesis that different aspects of BBB leakage can be measured with different sequences, each of which has its own strengths and limitations. These techniques may as such provide complementary information about BBB

leakage in the context of cSVD and dementia. While T1-weighted DCE-MRI with gadobutrol is suitable for measuring parenchymal BBB leakage, this technique is less suitable for measuring leakage of contrast agent into the CSF. Two problems that arise with T1-weighted imaging in CSF are the enhancement of highly vascularized structures such as the meninges and choroid plexus, and its relative insensitivity to low gadobutrol concentrations in CSF. In contrast, we have shown that detecting focal pericortical leakage of low concentrations of gadobutrol into the CSF is feasible with delayed post-contrast FLAIR imaging. Unlike T1-weighted DCE-MRI, the T2-weighted FLAIR sequence is insensitive to high contrast agent concentrations within blood, and more sensitive to low gadobutrol concentrations in CSF.

Despite the abovementioned important strengths, there are also limitations to measuring BBB leakage with MRI in memory clinic patients. For example, it remains impossible to assess the presence of amyloid- β and tau proteins using this technique. Additional more invasive procedures such as lumbar puncture or positron emission tomography (PET) are required to obtain such information, which places increasing burden on research participants. Combined PET-MRI systems may partly reduce participant burden while increasing the spatial compatibility between both modalities. However, the spatial resolution of *in vivo* MRI is limited, which is also related to the restricted ethical time frame during which one can subject a participant, especially patients, to MRI scanning for scientific purposes. Moving to higher field strengths (i.e., 7 Tesla) may increase the spatial resolution, but this will come at the cost of increased susceptibility to motion artifacts and field inhomogeneity problems. Regardless of time restrictions and field strength, it remains impossible to study the cerebral vasculature at the individual capillary level with MRI. The spatial resolution of amyloid-PET is also limited, and unfortunately it remains difficult to distinguish between amyloid plaques and CAA pathology⁵⁸. *Post mortem* MRI with subsequent histological examination of brain tissue partly overcomes these issues. Whole brain cerebrovascular burden can be mapped in detail with *post mortem* MRI⁵⁹, and subsequent histology can generate detailed information on the local severity of individual vascular damage and parenchymal amyloid- β . Therefore, we assessed BBB leakage of plasma proteins into the vessel walls and brain tissue of pathologically confirmed CAA cases in chapter 6. However, obvious limitations this study include restricted sample size due to the limited availability of *post mortem* tissue, limited histopathological coverage of the brain, and the lack of information about the clinical trajectories of these patients over the years prior to death.

Finally, there is a lack of studies describing the temporal evolution of BBB disruption in relation to AD biomarker progression and cerebrovascular lesion development. In chapter 4 we

demonstrated the chronic nature of pericortical leaks in normal aging and memory clinic patients using repeated assessments, though our sample size was very low ($n=3$). Longitudinal studies frequently form a challenge with regard to human studies because it is time consuming, expensive, and prone to participant drop-out, especially in individuals with neurodegenerative disease. Moreover, the recent discussion about possible adverse effects of gadolinium retention in the brain after multiple contrast injections poses ethical questions to this kind of research⁶⁰. Animal studies may form a complementary opportunity to address BBB development over time in cSVD and AD, though still, translating the findings in these models to humans is problematic.

Implications for clinical care and directions for future research

The scientific results presented in this thesis have several implications for clinical care, which will be presented in the following sections together with suggested directions for future research. Firstly, our findings in chapter 2, as well as earlier studies^{61,62}, suggest that individuals with multiple types of pathology are more vulnerable to neurodegeneration and cognitive decline compared to those with only a single type of pathology. While existing clinical criteria now consider extensive cerebrovascular pathology as an exclusion criterion for a diagnosis of MCI due to AD or a probable diagnosis of AD type dementia^{63,64}, making the binary distinction between MCI/vascular cognitive impairment and AD/vascular dementia may not reflect the actual multifactorial underlying pathology in many older individuals³. Adding a vascular component to the etiological profile classification system of individuals with cognitive impairment⁶³ may benefit the prognostic accuracy of the expected cognitive trajectories and rate of neurodegeneration. To achieve this, longitudinal studies in large cohorts with repeated MRI, neuropsychological testing, and AD biomarker assessment are needed to further determine the individual and additive/synergistic influence of AD and cSVD pathology on neurodegeneration and cognitive decline in (early) dementia. Such studies should preferably include individuals with suspected AD etiology based on clinical assessment as well as those with relatively large cerebrovascular disease burden and/or presumed vascular cognitive impairment to capture the full spectrum of AD and cSVD. Future studies and consensus meetings between scientific experts in the field should focus on the development of compatible imaging and pathological scales that capture as many vascular pathological aspects as possible (i.e., infarcts, white matter hyperintensities (WMH), hemorrhage, microvascular lesions), that could be implemented in the recently developed AD biomarker classification system for research purposes^{12, 65, 66}. Such a standardized vascular scale would enhance comparability between studies with improved study sample characterization across studies, although it might be a challenge to capture all aspects of cerebrovascular disease into a

simplified score. As a start, future studies should follow previously proposed imaging guidelines to measure cSVD to maximize standardization of the findings across studies ⁶⁷.

Secondly, our results in chapter 3 suggest that BBB leakage may contribute to cognitive decline through its effects on WMH pathology. This makes vascular integrity a potential target for the development of future treatment and prevention strategies that are aimed at slowing down cSVD progression and cognitive decline in memory clinic patients. Importantly, prevention strategies should be applied at an early stage in the disease process when brain damage can still be prevented. Accumulating evidence suggests that promoting vascular health in midlife by targeting common risk factors of cSVD and AD may reduce the risk of cognitive decline and dementia in late life ^{68,69}. Although currently no therapeutic prevention and/or treatment strategies for cSVD exist, a recent review has provided an overview of drugs which may have relevant properties to increase BBB integrity in cSVD and which are already licensed for other disorders ⁷⁰. The availability of such drugs provides an opportunity of drug repurposing. Measurement of BBB leakage with DCE-MRI or post-contrast FLAIR imaging may form a way to identify individuals at risk of cSVD-related brain abnormalities and to monitor treatment response in trials. Ideally, future studies should first focus on optimization of the DCE-MRI technique to increase its sensitivity. Although the technique in its current state can be applied to assess associations between regional BBB leakage and biomarkers or treatment response on a group level, its sensitivity at the individual level is insufficient because the technique is prone to noise. Moving to higher field strengths (i.e. 7 Tesla MRI) will likely increase the signal-to-noise ratio of the contrast leakage measurements, but this may not be feasible to implement in many universities and hospitals because of the unavailability and high costs of such scanners. Another recommendation for future studies that follows from the previous sections is that both cerebrovascular pathology and AD biomarkers (amyloid- β , tau, and neurodegeneration) should be taken into account when measuring BBB leakage. PET-MRI forms an interesting tool that enables the simultaneous assessment and localization of BBB leakage and amyloid- β deposition. Combining these techniques may enable future studies to determine the relative strength of the associations between BBB leakage, MRI markers of cerebrovascular damage, and AD biomarkers, and their spatial overlap. Such studies could also establish to what extent these types of pathology have independent or interactive effects on cognition. Moreover, longitudinal imaging studies that repeatedly measure BBB leakage and assess the development of AD biomarkers, cerebrovascular lesions, and cognitive decline over time are necessary to shed light on the temporal sequence of events. Combining such longitudinal assessments with *post mortem* histological examination of the same individuals may validate the *in*

in vivo measurements, though such studies will probably be hard to establish in practice.

Finally, the clinical relevance of pericortical BBB leaks that we identified on delayed post-contrast FLAIR images in memory clinic patients and cognitively normal individuals in chapter 4 needs further investigation. Longitudinal studies in larger study samples are warranted to examine whether these presumably chronic, focal pericortical leakage spots represent vessels that are prone to CMB development, as suggested in an earlier case study⁴¹, and whether they are associated with other MRI markers of cerebral pathology and cognitive decline. Such studies would be especially interesting to conduct in individuals with possible or probable CAA⁷¹, which are known to be at risk for lobar CMB development⁷². Our results in chapter 5 and chapter 6 support the idea that BBB leakage may form a mechanism underlying spontaneous hemorrhagic lesion development in populations with cSVD. Future studies should explore therapeutic possibilities to retain BBB integrity, such as the control of inflammatory processes⁷³, as treatment strategies aimed at reducing the risk of intracerebral hemorrhage and CMB development in CAA and hypertensive vasculopathy. Because currently no treatment strategies exist to treat or reduce the risk of cSVD-related cerebral hemorrhage, the development of successful treatment methods would be a substantial step forward in this field⁷⁴. As mentioned earlier, diminishing the measurement error in DCE-MRI would improve risk assessment and treatment response at the individual level.

Conclusion: small vessels matter

The studies in this thesis show that widespread damage to the extensive network of small vessels in the brain may have a significant impact on brain health and cognition in memory clinic patients and older individuals with normal cognition. We showed that BBB leakage forms a key mechanism in cSVD and AD type dementia through complex associations with cerebrovascular lesions and cognitive performance. Future longitudinal studies are needed to disentangle the complex relationship between BBB leakage, cSVD, AD pathology, and cognitive decline. Further improvement of the DCE-MRI method and the development of treatment strategies that target BBB integrity may form important steps towards identifying individuals at risk with the ultimate goal of delaying cerebrovascular lesion development and cognitive decline.

REFERENCES

1. Kester MI, Goos JD, Teunissen CE, et al. Associations between cerebral small-vessel disease and Alzheimer disease pathology as measured by cerebrospinal fluid biomarkers. *JAMA neurology* 2014;71:855-862.
2. Attems J, Jellinger KA. The overlap between vascular disease and Alzheimer's disease--lessons from pathology. *BMC medicine* 2014;12:206.
3. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* 2007;69:2197-2204.
4. Guzman VA, Carmichael OT, Schwarz C, Tosto G, Zimmerman ME, Brickman AM. White matter hyperintensities and amyloid are independently associated with entorhinal cortex volume among individuals with mild cognitive impairment. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2013;9:S124-131.
5. Park JH, Seo SW, Kim C, et al. Effects of cerebrovascular disease and amyloid beta burden on cognition in subjects with subcortical vascular cognitive impairment. *Neurobiology of aging* 2014;35:254-260.
6. Vemuri P, Lesnick TG, Przybelski SA, et al. Vascular and amyloid pathologies are independent predictors of cognitive decline in normal elderly. *Brain : a journal of neurology* 2015;138:761-771.
7. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *The Lancet Neurology* 2013;12:207-216.
8. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *The Lancet Neurology* 2010;9:119-128.
9. Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nature reviews Neuroscience* 2011;12:723-738.
10. Kalara RN, Akinyemi R, Ihara M. Does vascular pathology contribute to Alzheimer changes? *J Neurol Sci* 2012;322:141-147.
11. de la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. *The Lancet Neurology* 2004;3:184-190.
12. Jack CR, Jr., Bennett DA, Blennow K, et al. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* 2016;87:539-547.
13. Zekry D, Duyckaerts C, Moulia R, et al. Degenerative and vascular lesions of the brain have synergistic effects in dementia of the elderly. *Acta Neuropathol* 2002;103:481-487.
14. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nature reviews Neuroscience* 2006;7:41-53.
15. Abbott NJ. Blood-brain barrier structure and function and the challenges for CNS drug delivery. *Journal of inherited metabolic disease* 2013;36:437-449.
16. Tarasoff-Conway JM, Carare RO, Osorio RS, et al. Clearance systems in the brain-implications for Alzheimer disease. *Nature reviews Neurology* 2015;11:457-470.
17. Deane R, Wu Z, Sagare A, et al. LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. *Neuron* 2004;43:333-344.
18. Bading JR, Yamada S, Mackic JB, et al. Brain clearance of Alzheimer's amyloid-beta40 in the squirrel monkey: a SPECT study in a primate model of cerebral amyloid angiopathy. *J Drug Target* 2002;10:359-368.
19. Mawuenyega KG, Sigurdson W, Ovod V, et al. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science* 2010;330:1774.
20. van de Haar HJ, Burgmans S, Jansen JF, et al. Blood-Brain Barrier Leakage in Patients with Early Alzheimer Disease. *Radiology* 2016;152244.
21. Montagne A, Barnes SR, Sweeney MD, et al. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* 2015;85:296-302.
22. Starr JM, Farrall AJ, Armitage P, McGurn B, Wardlaw J. Blood-brain barrier permeability in Alzheimer's disease: a case-control MRI study. *Psychiatry research* 2009;171:232-241.
23. Schlageter NL, Carson RE, Rapoport SI. Examination of blood-brain barrier permeability in dementia of the Alzheimer type with [68Ga]EDTA and positron emission tomography. *J Cereb Blood Flow Metab* 1987;7:1-8.

24. Caserta MT, Caccioppo D, Lapin GD, Ragin A, Groothuis DR. Blood-brain barrier integrity in Alzheimer's disease patients and elderly control subjects. *The Journal of neuropsychiatry and clinical neurosciences* 1998;10:78-84.
25. Kay AD, May C, Papadopoulos NM, et al. CSF and serum concentrations of albumin and IgG in Alzheimer's disease. *Neurobiology of aging* 1987;8:21-25.
26. Frolich L, Kornhuber J, Ihl R, Fritze J, Maurer K, Riederer P. Integrity of the blood-CSF barrier in dementia of Alzheimer type: CSF/serum ratios of albumin and IgG. *European archives of psychiatry and clinical neuroscience* 1991;240:363-366.
27. Munoz DG, Erkinjuntti T, Gaytan-Garcia S, Hachinski V. Serum protein leakage in Alzheimer's disease revisited. *Ann N Y Acad Sci* 1997;826:173-189.
28. Rozemuller JM, Eikelenboom P, Kamphorst W, Stam FC. Lack of evidence for dysfunction of the blood-brain barrier in Alzheimer's disease: an immunohistochemical study. *Neurobiology of aging* 1988;9:383-391.
29. Erickson MA, Banks WA. Blood-brain barrier dysfunction as a cause and consequence of Alzheimer's disease. *J Cereb Blood Flow Metab* 2013;33:1500-1513.
30. Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease--systematic review and meta-analysis. *Neurobiology of aging* 2009;30:337-352.
31. Zhang CE, Wong SM, van de Haar HJ, et al. Blood-brain barrier leakage is more widespread in patients with cerebral small vessel disease. *Neurology* 2017;88:426-432.
32. Wardlaw JM. Blood-brain barrier and cerebral small vessel disease. *J Neurol Sci* 2010;299:66-71.
33. Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 2008;57:178-201.
34. Snowden DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. *Jama* 1997;277:813-817.
35. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke* 2011;42:2672-2713.
36. Xiong L, Reijmer YD, Charidimou A, Cordonnier C, Viswanathan A. Intracerebral hemorrhage and cognitive impairment. *Biochimica et biophysica acta* 2016;1862:939-944.
37. Latour LL, Kang DW, Ezzeddine MA, Chalela JA, Warach S. Early blood-brain barrier disruption in human focal brain ischemia. *Annals of neurology* 2004;56:468-477.
38. Keep RF, Zhou N, Xiang J, Andjelkovic AV, Hua Y, Xi G. Vascular disruption and blood-brain barrier dysfunction in intracerebral hemorrhage. *Fluids and Barriers of the CNS* 2014;11.
39. Merali Z, Huang K, Mikulis D, Silver F, Kassner A. Evolution of blood-brain-barrier permeability after acute ischemic stroke. *PloS one* 2017;12:e0171558.
40. Kidwell CS, Burgess R, Menon R, Warach S, Latour LL. Hyperacute injury marker (HARM) in primary hemorrhage: A distinct form of CNS barrier disruption. *Neurology* 2011;77:1725-1728.
41. van Nieuwenhuizen KM, Hendrikse J, Klijn CJM. New microbleed after blood-brain barrier leakage in intracerebral haemorrhage. *BMJ Case Rep* 2017;2017.
42. Fisher M. Cerebral Microbleeds and Thrombolysis: Clinical Consequences and Mechanistic Implications. *JAMA neurology* 2016;73:632-635.
43. Reijmer YD, van Veluw SJ, Greenberg SM. Ischemic brain injury in cerebral amyloid angiopathy. *J Cereb Blood Flow Metab* 2016;36:40-54.
44. Shi Y, Wardlaw JM. Update on cerebral small vessel disease: a dynamic whole-brain disease. *Stroke and vascular neurology* 2016;1:83-92.
45. Shi ZS, Duckwiler GR, Jahan R, et al. New Cerebral Microbleeds After Mechanical Thrombectomy for Large-Vessel Occlusion Strokes. *Medicine* 2015;94:e2180.
46. Dassan P, Brown MM, Gregoire SM, Keir G, Werring DJ. Association of cerebral microbleeds in acute ischemic stroke with high serum levels of vascular endothelial growth factor. *Arch Neurol* 2012;69:1186-1189.
47. Jeon SB, Kwon SU, Cho AH, Yun SC, Kim JS, Kang DW. Rapid appearance of new cerebral microbleeds after acute ischemic stroke. *Neurology* 2009;73:1638-1644.
48. Lange C, Storkebaum E, de Almodovar CR, Dewerchin M, Carmeliet P. Vascular endothelial growth factor: a neurovascular target in neurological diseases. *Nature reviews Neurology* 2016;12:439-454.
49. Ruiz de Almodovar C, Lambrechts D, Mazzone M, Carmeliet P. Role and therapeutic potential of VEGF in the nervous system. *Physiol Rev* 2009;89:607-648.

50. Zhang JB, Li MF, Zhang HX, et al. Association of serum vascular endothelial growth factor levels and cerebral microbleeds in patients with Alzheimer's disease. *European journal of neurology : the official journal of the European Federation of Neurological Societies* 2016.
51. Jellinger KA, Attems J. Prevalence and pathogenic role of cerebrovascular lesions in Alzheimer disease. *J Neurol Sci* 2005;229-230:37-41.
52. Maia LF, Mackenzie IR, Feldman HH. Clinical phenotypes of Cerebral Amyloid Angiopathy. *J Neurol Sci* 2007;257:23-30.
53. Thal DR, Griffin WST, de Vos RAI, Ghebremedhin E. Cerebral amyloid angiopathy and its relationship to Alzheimer's disease. *Acta Neuropathologica* 2008;115:599-609.
54. Rosenberg GA. Neurological diseases in relation to the blood-brain barrier. *J Cereb Blood Flow Metab* 2012;32:1139-1151.
55. Rosenberg GA. Blood-Brain Barrier Permeability in Aging and Alzheimer's Disease. *The journal of prevention of Alzheimer's disease* 2014;1:138-139.
56. Raja R, Rosenberg GA, Caprihan A. MRI measurements of Blood-Brain Barrier function in dementia: A review of recent studies. *Neuropharmacology* 2017.
57. Wong SM, Jansen JFA, Zhang CE, et al. Measuring subtle leakage of the blood-brain barrier in cerebrovascular disease with DCE-MRI: Test-retest reproducibility and its influencing factors. *Journal of magnetic resonance imaging : JMRI* 2017;46:159-166.
58. Charidimou A, Boulouis G, Gurol ME, et al. Emerging concepts in sporadic cerebral amyloid angiopathy. *Brain : a journal of neurology* 2017;140:1829-1850.
59. van Veluw SJ, Charidimou A, van der Kouwe AJ, et al. Microbleed and microinfarct detection in amyloid angiopathy: a high-resolution MRI-histopathology study. *Brain : a journal of neurology* 2016;139:3151-3162.
60. Kanal E, Tweedle MF. Residual or retained gadolinium: practical implications for radiologists and our patients. *Radiology* 2015;275:630-634.
61. Vemuri P, Lesnick TG, Przybelski SA, et al. Vascular and amyloid pathologies are independent predictors of cognitive decline in normal elderly. *Brain : a journal of neurology* 2015.
62. Lee MJ, Seo SW, Na DL, et al. Synergistic effects of ischemia and beta-amyloid burden on cognitive decline in patients with subcortical vascular mild cognitive impairment. *JAMA psychiatry* 2014;71:412-422.
63. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2011;7:270-279.
64. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2011;7:263-269.
65. Vemuri P, Knopman DS. The role of cerebrovascular disease when there is concomitant Alzheimer disease. *Biochimica et biophysica acta* 2016;1862:952-956.
66. Smallwood A, Oulhaj A, Joachim C, et al. Cerebral subcortical small vessel disease and its relation to cognition in elderly subjects: a pathological study in the Oxford Project to Investigate Memory and Ageing (OPTIMA) cohort. *Neuropathol Appl Neurobiol* 2012;38:337-343.
67. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *The Lancet Neurology* 2013;12:822-838.
68. Gottesman RF, Schneider AL, Zhou Y, et al. Association Between Midlife Vascular Risk Factors and Estimated Brain Amyloid Deposition. *Jama* 2017;317:1443-1450.
69. Launer LJ, Masaki K, Petrovitch H, Foley D, Havlik RJ. The association between midlife blood pressure levels and late-life cognitive function. *The Honolulu-Asia Aging Study. Jama* 1995;274:1846-1851.
70. Bath PM, Wardlaw JM. Pharmacological treatment and prevention of cerebral small vessel disease: A review of potential interventions. *International Journal of Stroke* 2015;10:469-478.
71. Knudsen KA, Rosand J, Karluk D, Greenberg SM. Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. *Neurology* 2001;56:537-539.

72. Greenberg SM. Cerebral amyloid angiopathy: prospects for clinical diagnosis and treatment. *Neurology* 1998;51:690-694.
73. Yan P, Zhu A, Liao F, et al. Minocycline Reduces Spontaneous Hemorrhage in Mouse Models of Cerebral Amyloid Angiopathy. *Stroke* 2015;46:1633-1640.
74. Qureshi AI, Mendelow AD, Hanley DF. Intracerebral haemorrhage. *Lancet* 2009;373:1632-1644.

ADDENDUM

SUMMARY.

SAMENVATTING.

KNOWLEDGE VALORIZATION.

DANKWOORD.

CURRICULUM VITAE.

LIST OF PUBLICATIONS.

THESIS DEFENCES FROM MHEENS.

SUMMARY

Late-onset Alzheimer disease (AD) is the most common cause of dementia and characterized by cerebral misfolded protein accumulation (amyloid- β plaques and tau tangles), neurodegeneration, and progressive cognitive decline. Multiple *post mortem* and magnetic resonance imaging (MRI) studies have shown that most older individuals with AD also exhibit signs of vascular pathology in the brain, which is frequently caused by cerebral small vessel disease (cSVD). The mechanisms underlying cSVD-related vascular damage and its role in AD are not well understood, and cerebrovascular pathology remains frequently ignored in research disease models and clinical criteria for AD. In this thesis we explored the associations between microvascular damage, markers of AD, and cognition, to expand current insights on vascular contributions to the pathophysiology and symptomatology of dementia.

In chapter 2 we explored the association between MRI-visible cSVD severity (i.e. white matter hyperintensity (WMH) volume) and neurodegeneration (i.e. hippocampal volume) in memory clinic patients with normal and abnormal amyloid- β levels using data from the multicenter LeARN study. The results demonstrated that cSVD severity was negatively associated with hippocampal volume in individuals without dementia with abnormal amyloid- β status, but this association was not present in those with normal amyloid- β status or in individuals with dementia. These findings suggest that cerebrovascular pathology is associated with neurodegenerative processes at an early stage of AD.

The next chapters in this thesis focused on the blood-brain barrier (BBB), which is a term that refers to the selective permeability properties of the cerebral blood vessels. BBB disruption (also referred to as BBB leakage) is considered to be the core mechanism underlying microvascular damage in cSVD, and previous studies suggest that BBB dysfunction also plays a role in AD.

In chapter 3 and chapter 4, two post-contrast MRI methods were used to measure BBB dysfunction in the form of contrast agent extravasation in older individuals within the range from normal cognition to AD-type dementia. In chapter 3, dynamic contrast-enhanced MRI was applied to measure BBB leakage within the cerebral tissue. We examined the cross-sectional relationship between BBB dysfunction, neurodegeneration (i.e., hippocampal volume), cSVD severity (i.e. WMH volume), and cognition. The results indicated a positive association between BBB dysfunction within the gray and white matter and cSVD severity across the whole study sample. Moreover, we found a negative association between BBB dysfunction within the white matter

tissue and information processing speed, which was mediated by cSVD severity. In chapter 4 we assessed BBB dysfunction using post-contrast FLAIR imaging, which is a relatively understudied technique that is highly sensitive to measure extravasation of contrast agent into the cerebrospinal fluid. We identified focal spots of pericortical signal enhancement in approximately 20% of cognitively normal individuals, and in 30-40% of individuals with cognitive impairment. This form of BBB dysfunction was associated with increasing age and signs of previous ischemic stroke on MRI. Interestingly, the focal contrast agent extravasation remained stable over time in three cases that came to follow-up after 21-22 months. The results of these studies demonstrate that subtle BBB leakage is probably a chronic process that is associated with cerebrovascular pathology and aging in memory clinic patients and cognitively normal older controls.

In chapter 5 and chapter 6 we investigated the association between BBB dysfunction and cSVD-related hemorrhagic pathology (cerebral microbleeds (CMB) and intracerebral hemorrhage (ICH)). Chapter 5 constitutes a systematic review on BBB dysfunction in the etiology of cSVD-related CMB and ICH. We searched Medline and Embase for human and animal studies reporting on BBB dysfunction in the context of ICH and/or CMBs. Signs of BBB dysfunction were found in the majority of the included studies (twenty out of twenty-five studies). This finding suggests that BBB integrity deserves further attention as a potential target for treatment strategies aimed at reducing the risk for spontaneous ICH. In chapter 6 we explored the association between BBB dysfunction and microvascular lesions in brain tissue of patients with cerebral amyloid angiopathy (CAA). In this *post mortem* MRI-histopathology study we identified a positive association between BBB dysfunction in the form of plasma protein extravasation and CAA severity. Furthermore, BBB dysfunction was associated with a higher number of CMBs identified on *post mortem* MRI.

Chapter 7 presents a discussion of the results in a more general context. The studies in this thesis show that widespread damage to the extensive network of small vessels in the brain can have a, possibly far underestimated, significant impact on brain health and cognitive performance in normal aging and in the context of the memory clinic. Research considerations, clinical implications, and suggested directions for future research are described.

SAMENVATTING

De ziekte van Alzheimer (AD) is de meest voorkomende vorm van dementie en wordt gekenmerkt door abnormale eiwit ophoping in de hersenen (amyloide plaques en tau vezelkluwens), neurodegeneratie, en progressieve cognitieve achteruitgang. Verschillende *post mortem* en magnetische resonantie imaging (MRI) studies hebben aangetoond dat de hersenen van de meeste oudere mensen met AD ook vasculaire pathologie bevatten, waar cerebrale microangiopathie (cSVD) vaak de oorzaak van is. Er is nog maar weinig bekend over de mechanismen die ten grondslag liggen aan cSVD-gerelateerde vasculaire hersenschade in AD, en cerebrovasculaire pathologie wordt vaak niet meegenomen in onderzoeksmodellen en klinische criteria voor AD. In dit proefschrift hebben we associaties onderzocht tussen (micro)vasculaire hersenschade, markers voor AD, en cognitie, om het inzicht in de rol van vaatschade in de pathofysiologie en symptomatologie van dementie te vergroten.

In hoofdstuk 2 hebben we data van de multi-centrum LeARN studie gebruikt om de associatie tussen MRI-zichtbare cSVD (i.e. witte stof hyperintensiteit volume) en neurodegeneratie (i.e. hippocampaal volume) te onderzoeken in geheugenpolipatienten met normale en abnormale amyloid- β levels (gemeten in de cerebrospinale vloeistof (CSF)). We vonden een negatieve associatie tussen cSVD en hippocampaal volume in mensen zonder dementie met abnormale amyloid- β waarden, maar deze associatie was niet aanwezig in personen met normale amyloid- β waarden of in mensen met dementie. Deze bevindingen wijzen erop dat neurodegeneratieve processen gerelateerd zijn aan cerebrovasculaire pathologie in een vroeg stadium van AD.

In de volgende hoofdstukken van dit proefschrift ligt de focus op de bloed-hersenbarrière (BBB). De term BBB refereert naar de selectieve doorlaatbaarheid van de bloedvaatjes in de hersenen. BBB disfunctie (ook wel BBB lekkage genoemd) wordt gezien als een onderliggend mechanisme van cSVD, en uit eerder onderzoek blijkt dat er ook een mogelijke associatie bestaat tussen BBB disfunctie en AD.

In hoofdstuk 3 en hoofdstuk 4 zijn twee post-contrast MRI technieken gebruikt om BBB disfunctie in de vorm van contrastlekkage te meten in oudere volwassenen met normale cognitie, een milde cognitieve beperking, of AD-type dementie. In hoofdstuk 3 hebben we gebruik gemaakt van dynamic contrast-enhanced MRI om BBB lekkage te meten in het hersenweefsel. We hebben de cross-sectionele relatie onderzocht tussen BBB lekkage, neurodegeneratie (i.e. hippocampaal volume), cSVD (i.e. witte stof hyperintensiteit volume), en cognitie. We vonden een positieve

associatie tussen BBB lekkage in zowel de grijze als witte stof en cSVD over de hele onderzochte groep. Daarnaast was er een negatieve associatie tussen BBB disfunctie in de witte stof en informatieverwerkingssnelheid, hetgeen werd beïnvloed door cSVD. In hoofdstuk 4 hebben we BBB lekkage gemeten met post-contrast FLAIR imaging, een techniek die in hoge mate gevoelig is om lekkage van contrastmiddel door de BBB in het CSF te identificeren. Met behulp van deze scan hebben we focale pericorticale lekkages gevonden in 20% van de personen met normale cognitie, en in 30-40% van de mensen met een cognitieve beperking. Deze vorm van BBB disfunctie kwam vaker voor bij personen met een hogere leeftijd en bij mensen met MRI-zichtbare ischemische infarcten. De focale contrastlekkage bleef stabiel over de tijd in drie gevallen die na 21-22 maanden opnieuw onderzocht werden. De resultaten van deze studies laten zien dat subtiele BBB lekkage waarschijnlijk een chronisch proces is dat gerelateerd is aan cerebrovasculaire pathologie, cognitieve achteruitgang, en veroudering in geheugenpolipatiënten en cognitief normale controles.

In hoofdstuk 5 en hoofdstuk 6 hebben we de associatie tussen BBB disfunctie en cSVD-gerelateerde bloedingen (cerebrale microbloedingen (CMB) en intracerebrale bloedingen (ICH) onderzocht. Hoofdstuk 5 omvat een systematisch literatuuronderzoek over BBB disfunctie in de etiologie van cSVD-gerelateerde CMB en ICH. Zowel dier- als mensenstudies die BBB disfunctie in de context van ICH en/of CMB hebben onderzocht werden geïdentificeerd via Medline en Embase. 20 van de 25 studies vonden tekenen van BBB disfunctie in dieren of mensen met cSVD-gerelateerde bloedingen. Deze bevinding wijst erop dat BBB integriteit meer aandacht verdient als mogelijk doelwit voor behandelstrategieën die gericht zijn op het verkleinen van het risico op spontane ICH. In hoofdstuk 6 hebben we de associatie tussen BBB disfunctie en microvasculaire lesies in hersenweefsel van patiënten met cerebrale amyloid angiopathie (CAA) onderzocht. In deze *post mortem* MRI-histopathologie studie vonden we een positieve associatie tussen BBB disfunctie in de vorm van plasma eiwit lekkage en CAA. Daarnaast was BBB disfunctie geassocieerd met een hoger aantal microbloedingen op post mortem MRI.

Hoofdstuk 7 presenteert een discussie van de resultaten in een algemene context. De studies in dit proefschrift laten zien dat wijdverspreide schade aan het netwerk van kleine bloedvaatjes in de hersenen een, tot op heden mogelijk onderschatte, significante impact heeft op de conditie van hersenen en cognitie in normale veroudering en in patiënten van de geheugenpolikliniek.

KNOWLEDGE VALORIZATION

The general aim of this thesis was to investigate whether microvascular dysfunction in the form of blood-brain barrier (BBB) disruption is associated with cerebrovascular lesions and common markers of Alzheimer disease (AD), and how these markers are associated with cognitive performance in memory clinic patients and older individuals with normal cognition. This valorization paragraph describes the societal relevance and potential implementation opportunities of the results.

Relevance

Dementia is a major cause of progressive cognitive and functional decline in older people and as such forms a serious threat to individual and public health. Around 40 million people are currently living with dementia, and the largest risk factor for the disease is age. With the increasing life expectancy of populations all over the world, the number of people with dementia is expected to double over the next 20 years. Alzheimer disease (AD) is the most common cause of dementia, contributing to an estimated 70% of cases. Importantly, although scientific advances over the past decades have significantly increased our understanding of the biological processes underlying AD, all clinical trials that target amyloid deposits have failed and there is currently no effective treatment available that delays the onset or slows down the progression of the disease.

One of the major challenges with regard to the development of AD treatment strategies is the multifactorial and heterogeneous nature of the disease. The key neuropathological hallmarks of AD include amyloid plaques and neurofibrillary tangles, but their pathophysiological cause remains unknown and the current view is that there is likely more than one factor contributing to the abnormal protein accumulation and associated cognitive decline. In this light, there is growing evidence that cerebrovascular damage plays a critical role in the development and progression of the disease. AD and vascular disease share many risk factors and multiple neuropathological and neuroimaging studies have shown that mixed brain pathology (neurodegenerative and vascular) is the most frequent finding in cases with dementia. A better understanding of the role of cerebrovascular damage in AD will probably facilitate the future development of successful healthcare interventions aimed at halting disease progression.

The studies in this thesis focus on the most frequent cause of cerebrovascular pathology in older individuals with AD, which is cerebral small vessel disease (cSVD) due to hypertensive vasculopathy (HV) and/or cerebral amyloid angiopathy (CAA). Previous research suggests that an

important underlying mechanism of both types of cSVD is blood-brain barrier (BBB) disruption, which refers to the failure of strict transport regulation from blood to brain and vice versa at the capillary level. BBB disruption may lead to leakage of toxic plasma components which probably results in cellular damage. Dysfunction of the BBB may at the same time disrupt waste product clearance from the brain. However, the extent of BBB dysfunction and associated pathology in aging and AD remains poorly understood. The studies in this thesis aid in clarifying the role of BBB leakage in cSVD and AD and provide basic insights into the possible clinical and pathological consequences of cSVD-related BBB leakage in normal aging and in memory clinic patients.

Main findings

The findings in the present thesis suggest that damage to the microvascular network in the brain negatively and independently affects brain health. More specifically, the research in this thesis suggests that dysfunction of the BBB along the continuous cerebrovascular endothelium in the brain is related to imaging markers of ischemic and hemorrhagic cerebrovascular brain damage and slower cognitive performance in individuals with and without cognitive impairment.

In the first study described in this thesis we found that cSVD severity is related to hippocampal neurodegeneration in individuals with early AD, but not in individuals with dementia or in the absence of cerebral amyloid pathology. This finding suggests that cerebrovascular disease is associated with neurodegenerative processes at an early disease stage when the brain is vulnerable due to co-existent AD pathology.

Next, we measured BBB dysfunction in living humans using two different magnetic resonance imaging approaches. With dynamic contrast-enhanced MRI we identified a positive association between BBB leakage in the cerebral gray and white matter and cSVD severity, which was in turn associated with a decline in processing speed performance in memory clinic patients and cognitively normal older individuals. With delayed post-contrast FLAIR imaging we identified small pericortical leaks of contrast agent into the cerebrospinal fluid in approximately 20-40% of the investigated study population. This type of BBB dysfunction was positively associated with age and MRI signs of old ischemic stroke. These findings demonstrate that subtle age- and/or cSVD related BBB dysfunction can be measured with these two MRI techniques, and provide novel insights into the role of BBB dysfunction in relation to cerebrovascular pathology and cognitive decline in older individuals.

Finally, we explored the association between hemorrhagic brain pathology and BBB dysfunction in a systematic review analysis and a *post mortem* MRI-histopathology study. In these studies we found support for the hypothesis that cSVD-related BBB dysfunction is a possible mechanism involved in the etiology of cerebral microbleed and intracerebral hemorrhage formation.

Target groups and implementation opportunities

The results of this thesis are of direct interest to researchers that examine the role of microvascular disruption in dementia and researchers in the cSVD and AD field in general. Our findings suggest that cerebrovascular disease can independently contribute to neurodegenerative processes at an early AD stage, which underlines the importance of incorporating cerebrovascular damage when studying AD. Moreover, the results in this thesis show that BBB leakage of contrast agent throughout the cerebral tissue and at small pericortical foci is associated with ischemic and hemorrhagic cerebrovascular damage and slower information processing speed in memory clinic patients and cognitively normal individuals. These findings expand current insights into the role of cerebrovascular damage in older individuals with and without cognitive impairment and open doors to further research on this topic

The results are also of direct interest to neuroradiologists that encounter post-contrast enhancement on FLAIR images in older individuals without a known neurological cause of BBB disruption. Our results suggest that contrast agent leakage into the CSF is probably common in older individuals with and without cognitive impairment, and as such should not be confused with severe conditions that may cause pericortical enhancement such as meningeal metastasis.

Also, the results in this thesis can be of interest to pharmaceutical companies who seek to find treatment strategies and biological (imaging) readouts with the aim of reducing cerebrovascular brain pathology and slowing down cognitive decline. Enhancing BBB integrity may form a possible target for future treatment that could slow down processing speed deterioration and cSVD-related brain damage. This will be relevant for individuals with AD and cSVD, but also to healthy older individuals at risk for cerebrovascular brain pathology due to age-associated BBB leakage. Should future research establish a cause-effect relationship between BBB leakage and hemorrhagic brain pathology, then the DCE-MRI and post-contrast FLAIR imaging techniques could be helpful for the identification of individuals at risk for hemorrhagic brain pathology and in the assessment of treatment response in future trials that target BBB integrity.

Innovation and future directions

The research described in this thesis further establishes a significant role of cerebrovascular abnormalities in AD, which has been identified in previous neuropathological and imaging studies. We expand this knowledge by showing that cSVD is an independent contributor to neurodegeneration in individuals with early AD. Moreover, we used two contrast-enhanced MRI techniques that are still very new in the aging and dementia research field. We applied dynamic contrast-enhanced MRI in individuals with and without cognitive impairment and for the first time performed BBB leakage analyses in the brain tissue with almost whole brain coverage, relating this to performance on tests for specific cognitive domains. In addition, we were the first to describe the appearance of pericortical enhancement on post-contrast FLAIR images in a substantial proportion of individuals with mild cognitive impairment and dementia, and to demonstrate pilot data showing that these small foci of contrast agent extravasation are of chronic nature in this study population. Furthermore, we summarized the currently available literature on BBB leakage as a possible important factor in the etiology of cSVD-related brain hemorrhage and contribute empirical evidence of an association between cerebral microbleeds and BBB leakage in *post mortem* tissue of CAA patients.

Before the results in this thesis can be of practical use, future research is necessary to further disentangle the complex associations between BBB dysfunction, cerebrovascular brain pathology, neurodegenerative pathology and cognition in older individuals. Longitudinal studies in larger samples that measure BBB dysfunction, cSVD markers, neurodegeneration, cognitive performance and amyloid pathology concurrently are needed to shed light on these complex associations and the temporal sequence of pathological events. Moreover, longitudinal studies that monitor BBB dysfunction with MRI in populations at risk for future hemorrhage, such as individuals with CAA, will shed light on the utility of BBB dysfunction measured with contrast-enhanced MRI as a marker for hemorrhage risk and as future target for the development of hemorrhage prevention strategies. Further refinement of the DCE-MRI technique would be of great interest since the technique in its current state is prone to noise and can be applied only to monitor subtle BBB leakage in subject groups but not at the individual level.

DANKWOORD

De afgelopen vier jaar zijn voorbij gevlogen, en het doorlopen van dit promotietraject was een fantastische ervaring! Dit proefschrift zou natuurlijk niet tot stand zijn gekomen zonder het werk en de steun van velen, en daarom wil ik verschillende mensen graag bedanken.

Allereerst gaat mijn dank uit naar mijn promotieteam. Wat heb ik veel van jullie geleerd!

Prof. Verhey, beste Frans, hartelijk dank voor jouw hulp en begeleiding, en voor het feit dat je me altijd gesteund hebt in mijn ambities. Jouw klinische en wetenschappelijke expertise vormen een sterke basis waar iedere promovendus op kan bouwen. Ik bewonder de bijzonder prettige sfeer die jij weet te creëren op de afdeling, het is geweldig om in jouw Alzheimer Centrum Limburg team te mogen werken.

Prof. Backes, beste Walter, het was een eer om met je te mogen samenwerken. Ik heb genoten van onze wetenschappelijke discussies en natuurlijk jouw altijd leerzame MRI-uitleg. Het plezier waarmee je je inzet voor de wetenschap en jouw betrokkenheid bij de professionele en persoonlijke ontwikkeling van jouw promovendi zijn bewonderenswaardig. Iedere promovendus die in jouw team terecht komt mag van geluk spreken met jou als begeleider.

Dr. Jacobs, beste Heidi, wat een geluk dat jij mijn copromotor wilde worden! Als mede neuroscience fanaat deel jij mijn passie voor het hersenonderzoek als geen ander. Jij stond altijd voor me klaar, of het nu was voor persoonlijk advies of voor een statistisch R recept, en ik kan niet beschrijven hoe fijn dat was! Jouw enthousiasme werkt aanstekelijk en ik heb de afgelopen jaren ontzettend veel van je geleerd. Hopelijk kunnen we onze samenwerking nog lang voortzetten!

De leden van de leescommissie, prof. Hofman, prof. Aldenkamp, prof. Biessels, prof. Hendrikse, en prof. Linden wil ik bedanken voor het lezen en beoordelen van mijn proefschrift.

Alle proefpersonen en hun naasten die hebben deelgenomen aan de studie 'vaatconditie bij dementie', bedankt voor jullie inzet en betrokkenheid. Jullie gaven me de energie om dit onderzoek tot een succes te brengen! Via deze weg wil ik ook Alzheimer Nederland en de Stichting 2Bike4Alzheimer bedanken voor het mogelijk maken van dit onderzoek.

Dr. Burgmans en dr. Jansen, beste Saartje en Jaap, hartelijk dank voor jullie bijdrage aan dit proefschrift. Saartje, zonder jou was dit project er niet geweest! Bedankt voor jouw vertrouwen in mij, en voor het delen van de bijzondere 400km fietservaring bij 2bike4Alzheimer. Jaap, bedankt voor de vele uurtjes die je vrij hebt gehouden om aan te schuiven bij het promotie overleg.

Prof. Klijn, beste Karin, bedankt voor jouw begeleiding en geduld bij het schrijven van het systematisch review. Het uiteindelijke resultaat heeft lang op zich laten wachten, maar ik ben trots op dit mooie hoofdstuk in mijn proefschrift.

Prof. Greenberg and prof. Bacskaï, dear Steve and Brian, thank you for giving me the opportunity to participate in your amazing research team. Maria, Markus, Jose, Guillaume, Wilmar and others, thanks for the great time in Boston! Susanne, het was een waar genoegen (lees: ‘excellent...’) om met jou te mogen werken in het lab en daarbuiten tijd met je door te brengen in Boston. Bedankt voor je enthousiasme en alles dat je me geleerd hebt, het was fantastisch!

Dr. Hoff, beste Erik, bedankt voor jouw praktische en inhoudelijke bijdrage aan dit proefschrift. Tiny Simons en Bjorn van Twillert, bedankt voor de hulp bij de inclusie en het delen van de neuropsychologie data vanuit Heerlen.

All co-authors of the chapters in this thesis, thank you for your valuable contribution to this work.

Danique en Olin, zonder jullie was de inclusie van meer dan 100 mensen waarschijnlijk niet gelukt! Bedankt voor jullie inzet ten behoeve van dit project. Inge, je begon als stagiaire bij ons op de afdeling en hebt geholpen tijdens de start van de inclusie, bedankt daarvoor! Rebecca, dankjewel voor jouw inzet tijdens de zomermaanden! Roy, bedankt voor jouw hulp en inzet bij de follow-up inclusie, die kwam op precies het goede moment! Joost, bedankt voor het werk dat je tijdens je stage verricht hebt.

De laboranten van de radiologie afdeling in the MUMC, bedankt voor jullie onmisbare bijdrage aan de data acquisitie en de vele gezellige uurtjes die we achter de scanner hebben gespendeerd. En speciale dank aan Roland, Guy en Sonja, jullie zijn toppers! Miguel, dank voor jouw betrokkenheid bij het BBB project! Ook de klinisch fysici (to be) waarmee ik heb mogen samenwerken, bedankt! Roald, wat ben je toch een fijn persoon. Bedankt voor jouw inzet tijdens ons pcFLAIR project, ik heb veel van je geleerd en met jou in de buurt was het altijd gezellig! En

daar hoort Eveline natuurlijk ook bij, bedankt voor je support en de gezelligheid op o.a. de zeilboot en bij ons kamelenvoortuig! Thomas, bedankt voor jouw bijdrage aan de diffusie data analyse tijdens jouw onderzoeksproject in het MUMC. David, ik kijk uit naar de voortzetting van ons project, we gaan er iets moois van maken! Monique, bedankt voor het regelen van vele (soms lastig in te plannen) afspraken en meer!

Alle radiologie collega's van 'de gang', bedankt! Joost, wat ben ik blij dat jij het team kwam versterken! Bedankt voor jouw bijdrage aan het DCE stuk en voor de gezelligheid op de werkvloer. Harm, jij hebt de basis gelegd voor mijn studie en was mijn tandem-buddy, bedankt voor jouw hulpvaardigheid en steun. En ook Marieke, Laura, Gerald, Lianne, Tamar en May, bedankt voor alle gezelligheid, ik kijk uit naar toekomstige spelletjes avonden en pubquizzes! Jos en Jan, jullie waren onmisbaar als ik het weer eens stuk had gemaakt, of het nou om de computer ging of om mijn fietsband. Bedankt voor jullie geduld en hulp.

Ook alle collega's van de Neuropsychologie afdeling wil ik bedanken voor de fijne sfeer op de werkvloer. Danielle, Astrid, Els, Nico en Ron, bedankt voor jullie persoonlijke betrokkenheid en onvermoeibare hulp! Dr. Gronenschild, beste Ed, zelfs tijdens jouw pensioen zette jij je nog in voor dit onderzoek, dat is toch eigenlijk veel te gek. Bedankt voor al jouw hulp en persoonlijke interesse. Tom en Ankie, bedankt dat ik altijd bij jullie kon binnenlopen voor hulp en advies. Babette, Rosalie, Angelique en Lisa, op de werkvloer, in de trein, op congres, tijdens het sporten of op het terras, met jullie was het altijd gezellig! Nikos, thanks for your support!

Dan de buurvrouwen en Hoezo? Club leden: Willemijn, Joany, Syenna, Elles, Danique en Lotte, zonder jullie waren de afgelopen jaren een stuk minder leuk geweest! Lieve Willemijn, wat fijn dat je zo vaak in de buurt was. Zonder dat je het weet heb je me veel rust bezorgd in tijden dat ik me druk maakte. Bedankt voor de fijne koffiemomentjes in Eindhoven en jouw steun de afgelopen jaren. Lotte, van jou word ik gewoon altijd vrolijk, wat ben je toch een heerlijk veelzijdig en ondernemend persoon! Bedankt voor je support en altijd eerlijke advies. Syenna, mijn lieve Limburg vriendin, bedankt voor jouw heerlijke kookkunsten (cho oishii, cho atsui desu ne?!), humor, en liefde. Ik vind het geweldig knap dat het je is gelukt om het proefschrift af te krijgen ondanks alles, en weeeeheeeeel, je bent een topper! Elles, lieve UNS40 buurvrouw en treinbuddy, het was super fijn om bij jou en Danique binnen te kunnen lopen voor een update over de nieuwste ontwikkelingen op de afdeling en onze weekendactiviteiten. Gelukkig blijven we voorlopig nog

even buurtjes! Lieve Joany, bedankt voor de gezelligheid op het werk, tijdens de hardloopsessies, en bij de vele etentjes en borrels.

Lieve Isabelle, wij hebben de afgelopen jaren bijna meer tijd doorgebracht met elkaar dan met onze mannen! Bij jou kon ik altijd met alles terecht, voor goed en eerlijk advies, een luisterend oor naar mijn onnozele verhalen, en uiteraard voor een goede grap. Het was geweldig om samen dit PhD avontuur te doorlopen, en ik ben ontzettend blij met onze vriendschap die daaruit ontstaan is!

Alieske en Marjosse, mijn blonde paranimfen, wat ben ik blij dat jullie achter mij staan tijdens de verdediging! Lieve Alieske, wat ben je toch een doorzetter en een sociaal persoon! We hebben ontzettend veel gedeeld de afgelopen jaren, en daarom zal onze vriendschap altijd bijzonder blijven. Bedankt dat Maastricht dankzij jou nog steeds als thuis voelt. Lieve Marjosse, Gertje, al meer dan 3 lustra hebben we erop zitten, en wat heb ik een geluk met jou als vriendin! Met jou kan ik lachen en huilen (en huilen van het lachen) en je staat altijd voor me klaar. Dankjewel voor jouw enthousiasme en je steun, ik kijk er nu al naar uit hoe wij later als 90+ers bekend staan als het beruchte duo!

Lieve Jeanne, mijn reisbuddy, studiegenootje, en super vriendin, wat een geluk dat wij onze gelijkenissen hebben ontdekt bij IBV en de coca-cola light automaat op het FSW! Bedankt dat je altijd een luisterend oor had voor mijn proefschrift verhalen. Hier is ie dan! Na >100miljoen p-500 responsen en de nodige insula activiteit ;). Lieve Tjerk&Nicole, onze trouwste Eindhoven logeerders, bedankt dat jullie altijd voor me klaar staan. Ik kijk uit naar onze toekomstige dubbel-dates! Lieve Mirjam, vanuit Utrecht vertrokken we naar Groningen en Maastricht, maar onze vriendschap weet die afstand gelukkig te overbruggen. Sven&Bri, mede Eindhovenaren en alpaca fans, met jullie valt er altijd wel wat te beleven. Bedankt voor de gezellige etentjes en uitjes! Lieve Ernst&Marja, bedankt dat jullie er altijd voor me zijn, onze vriendschap is me zeer dierbaar.

Lieve Achterhoekers, bedankt voor alle gezelligheid die jullie brachten de afgelopen jaren en de fantastische wintersport- en kampeertrips, jullie zijn Wunderbar!

Mijn allerliefste broertjes en zusjes, Mikie, Cher, Jesse en Chrissy, wat fijn dat jullie er zijn! Ik ben super trots op jullie en het is geweldig om met jullie op stap te gaan (en heel hard liedjes mee te zingen in de auto!). Ik hoop op nog heel veel toekomstige logeerpartijen! Lieve Harry, hopelijk

vind je het af en toe wel gezellig als het huis weer eens geterroriseerd wordt door je nepdochter ;) Bedankt voor de steun en interesse die je inmiddels al heel veel jaren aan ons geeft. Lieve Monique, met jou in huis (en op vakantie!) is het altijd gezellig, bedankt voor jouw lieve persoonlijkheid en enthousiasme. Lieve oma Vrony, bedankt voor al uw liefde (emmers vol!), en wat kunnen we altijd samen lachen! U heeft voor altijd een speciaal plekje in mijn hart. Lieve opa Leo, u bent een sterke rots in de branding (waar een geweldige dosis humor achter schuilt!) en een groot voorbeeld voor mij. Bedankt voor alle gezellige momenten en interessante gesprekken.

Ton, je bent de liefste schoonvader die ik me kan wensen! Vanaf mijn eerste rondleiding door de tuin voelde ik me thuis in het 'verre Oosten'. We hebben veel meegemaakt de afgelopen vier jaar, en ik bewonder jouw zorgzaamheid en doorzettingsvermogen. Wat fijn dat je Alies nu aan je zijde hebt.

Lieve mam, moesje, moose, jij kent me het beste van iedereen en je zou me zonder twijfel komen opzoeken op de maan (of Australië, of Boston (yeah, this is where the mit is!)). Dankjewel voor jouw onvoorwaardelijke liefde, je steun, en onze fantastische humor. Lieve pap, jij bent de ultieme 'family-man'. Bedankt voor jouw heerlijke kookkunsten, onze goede gesprekken onder het genot van een wijntje, en alle wijsheden die je me geleerd hebt door de jaren heen (er is altijd een weg!).

Lieve Thijs, jij bent de leukste en de liefste! Vlak voor mijn verhuizing naar Maastricht ontmoette ik jou, en wie had gedacht dat je een paar jaar later met deze gekke stresskipie een alpaca- en kamelen boerderij met John Deere op de planning zou hebben staan ;)! Dankjewel dat je er altijd voor me bent, en voor jouw oprechte interesse, humor, en alles wat je geeft. Ik heb ontzettend veel zin in alle avonturen die nog op ons wachten!

CURRICULUM VITAE

Whitney Freeze was born on May 25th, 1990 in Leiderdorp, the Netherlands. She attended secondary school at Bonaventuracollege Burggravelaan in Leiden, from which she graduated in 2008. After obtaining her BSc degree in Psychology at Leiden University (2011) she successfully applied to the research master Neuroscience & Cognition at Utrecht University. In the first year she started an 11-month internship at the Neurology department of Utrecht University Medical Center, under supervision of prof. dr. Geert Jan Biessels and dr. Yael Reijmer. During this internship she analyzed diffusion tensor images to investigate white matter integrity in patients with lacunar infarcts, and this work resulted in an authorship on a scientific publication. In the second year of the program she worked for six months as a visiting student at the Australian Imaging, Biomarkers and Lifestyle study at Melbourne University, Australia. Under supervision of prof. dr. Paul Maruff she studied the relationship between hippocampal volume, amyloid-beta levels, and cognitive performance in cognitively normal older individuals. After obtaining her MSc degree in 2014 she started a PhD project at the departments of Neuropsychology & Psychiatry and Radiology at Maastricht University, under supervision of prof. dr. Frans Verhey, prof. dr. ir. Walter Backes, and dr. Heidi Jacobs, which resulted in this thesis. During her PhD project she performed several teaching activities and presented her work at national and international conferences. In addition, she visited the Neurology Department at Massachusetts General Hospital, Harvard Medical School in Boston, USA, for four months to perform an MRI-histopathology study on blood-brain barrier leakage in cerebral amyloid angiopathy under supervision of prof. dr. Steven Greenberg, prof. dr. Brian Bacskai, and dr. Susanne van Veluw. After defending her thesis Whitney will continue working as postdoctoral researcher at the department of Neuropsychology & Psychiatry of Maastricht University.

LIST OF PUBLICATIONS

This thesis

- Freeze WM**, Jacobs HIL, Gronenschild EHBM, Jansen JF, Burgmans S, Aalten P, Clerx L, Vos SJ, van Buchem MA, Barkhof F, van der Flier WM, Verbeek MM, Rikkert MO, Backes WH, Verhey FR; LeARN project. White matter hyperintensities potentiate hippocampal volume reduction in non-demented older individuals with abnormal amyloid- β . *Journal of Alzheimer's Disease* 2017;55(1):333-342. doi:10.3233/JAD-160474.
- Freeze WM**, Jacobs HIL, de Jong JJ, Verheggen ICM, Gronenschild EHBM, Palm WM, Hoff EI, Wardlaw JM, Jansen JF, Verhey FR and Backes WH. On the link between blood-brain barrier leakage, white matter hyperintensities, neurodegeneration, and cognition. *Submitted*.
- Freeze WM**, Schnerr RS, Palm WM, Jansen JF, Jacobs HIL, Hoff EI, Verhey FR, Backes WH. Pericortical enhancement on delayed postgadolinium fluid-attenuated inversion recovery images in normal aging, mild cognitive impairment, and Alzheimer disease. *American Journal of Neuroradiology* 2017;38(9):1742-1747. doi: 10.3174/ajnr.A5273.
- Freeze WM**, Jacobs HIL, Schreuder FHBM, van Oostenbrugge RJ, Backes WH, Verhey FR, Klijn CJM. Blood-brain barrier dysfunction in small vessel disease related intracerebral hemorrhage – a systematic review. *Submitted*.
- Freeze WM**, Bacskai BJ, Frosch MP, Jacobs HIL, Backes WH, Greenberg SM, van Veluw SJ. Blood-brain barrier leakage and microvascular lesions in cerebral amyloid angiopathy: a *post mortem* MRI-histopathology study. *In preparation*.

Other

- Reijmer YD, **Freeze WM**, Leemans A, Biessels GJ; Utrecht Vascular Cognitive Impairment Study Group. The effect of lacunar infarcts on white matter tract integrity. *Stroke* 2013;44(7):2019-21. doi: 10.1161/STROKEAHA.113.001321.
- Riphagen JM, Gronenschild EHBM, Salat DH, **Freeze WM**, Ivanov D, Clerx L, Verhey FRJ, Aalten P, Jacobs HIL. Shades of white: diffusion properties of T1 and FLAIR defined white matter signal abnormalities differ in stages from cognitively normal to dementia. *Neurobiology of Aging* 2018;68:48-58. doi: 10.1016/j.neurobiolaging.2018.03.029.
- Douven E, Staals J, Schievink S, **Freeze WM**, Hellebrekers D, Wolz R, van Oostenbrugge RJ, Verhey FRJ, Kohler S, Aalten P. The role of MRI markers of lesion-related and generalized brain pathology in the development of post-stroke apathy and depression: results of the CASPER study. *Submitted*.
- Schievink SHJ, Douven E, Aalten P, Staals J, **Freeze WM**, Hellebrekers D, Wolz R, Jansen JF, van Oostenbrugge RJ, Verhey FRJ, Kohler S. The association of white matter hyperintensity volume, hippocampal volume, and global atrophy with post-stroke cognition. *Submitted*.

THESIS DEFENCES FROM MHENS – SCHOOL FOR MENTAL HEALTH AND NEUROSCIENCE

2013

Rob Havermans: **Bipolar disorder in daily life; Mood and cortisol responses to naturally occurring events.** Supervisor: Prof.dr. M. de Vries; Co-Supervisor: Dr. N. Nicolson.

Véronique Moers-Hornikx: **Deep brain stimulation and the cerebellum.** Supervisors: Prof.dr. J. Vles / Prof.dr. Y. Temel; Co-Supervisor: Dr. G. Hoogland.

Nicole Veldhorst-Janssen: **Intranasal delivery of rapid acting drugs.** Supervisors: Prof.dr. M. Marcus / Prof.dr. C. Neef; Co-Supervisor: Dr. P.H. van der Kuy.

Stéphanie Knippenberg: **Vitamin D and Multiple Sclerosis: immunological and clinical outcome.** Supervisor: Prof.dr. J. Cohen-Tervaert; Co-Supervisors: Dr. J. Damoiseaux / Dr. Y. Bols.

Erik D. Gommer: **Dynamic Cerebral Autoregulation: from methodology towards clinical application.** Supervisors: Prof.dr. W.H. Mess / Prof.dr. R.B. Panerai, UK; Co-Supervisor: Dr.ir. J.P.H. Reulen.

Olga A.H. Reneerkens: **Can PDE inhibition improve cognition ?** Translational insights. Supervisor: Prof.dr. H.W.M. Steinbusch; Co-Supervisor: Dr. J. Prickaerts.

Lyzel S. Elias-Sonnenschein: **Clinical and biomarker correlates of genetic risk factors for Alzheimer's disease.** Supervisor: Prof.dr. F.R.J. Verhey; Co-Supervisor: Dr. P.J. Visser.

Diego F. Mastroeni: **Epigenetic Dysregulation and the Pathophysiology of Alzheimer's Disease.** Supervisors: Prof.dr. H.W.M. Steinbusch / Prof.dr. P.D. Coleman, Sun City, Arizona; Co-Supervisors: Dr. B.P.F. Rutten / Dr. D.L.A. van den Hove.

Leonidas Chouliaras: **Epigenetic Regulation in Aging and Alzheimer's disease: A translational perspective.** Supervisor: Prof.dr. H.W.M. Steinbusch; Co-Supervisors: Dr. B.P.F. Rutten / Dr. D.L.A. van den Hove.

Liesbeth Knaepen: **Perinatal events and altered pain sensitivity in later life.** Supervisors: Prof.dr. E.A.J. Joosten / Prof.dr. D. Tibboel, EUR; Co-Supervisor: Dr. J. Patijn.

Marisela Martinez-Claros: **Hippocampal plasticity and corticosterone: From dendrites to behaviour.** Supervisor: Prof.dr. H.W.M. Steinbusch; Co-Supervisors: Dr. J.L. Pawluski / Dr. J. Prickaerts.

Marcus D. Lancé: **A circle of improvement in bleeding management: from laboratory to clinic and back.** Supervisors: Prof.dr. M.A.E. Marcu / Prof.dr. J.W.M. Heemskerk; Co-Supervisor: Dr. Y.M.C. Henskens.

Hilde Braakman: **Imaging the brain; neuronal correlates of cognitive impairment in children with frontal lobe epilepsy.** Supervisors: Prof.dr. A.P. Aldenkamp / Prof.dr. J.S.H. Vles; Co-Supervisors: Dr.ir. W.H. Backes / Dr. P.A.M. Hofman.

Willem H. van Zwam: **Aneurysmal subarachnoid hemorrhage: imaging strategies and cost-effectiveness aspects in diagnostic work-up and post-therapeutic follow-up.** Supervisors: Prof.dr. J.T. Wilmink / Prof.dr. J.E. Wildberger; Co-Supervisor: Dr. P.A.M. Hofman.

Klara De Cort: **The Pathogenesis of Panic Disorder.** Supervisors: Prof.dr. I. Myin-Germeys / Prof.dr. E.J.L. Griez; Co-Supervisors: Dr. K.R.J. Schruers / Dr. I. Van Diest, Leuven.

Kim van Wijck: **Mind the Gap; experimental studies on splanchnic hyperfusion and gastrointestinal integrity loss in man.** Supervisors: Prof.dr. W.A. Buurman / Prof.dr. C.H.C. Dejong; Co-Supervisor: Dr. K. Lenaerts.

Yvette Roke: **Antipsychotic-induced hyperprolactinemia in children and adolescents with mainly autism spectrum disorders. Prevalence, symptoms, clinical consequences and genetic risk factors.** Supervisors: Prof.dr. P.N. van Harten / Prof.dr. J.K. Buitelaar (RUN); Co-Supervisor: Dr. A. Boot (UMCG).

Fleur Goezinne: **Retinal detachment surgery: pre and postoperative prognostic factors.** Supervisors: Prof.dr. F. Hendrikse / Prof.dr. C.A.B. Webers; Co-Supervisor: Dr. E.C. La Heij (Amsterdam).

Ralph L.J.G. Maassen: **The Merits of Videolaryngoscopy during Glottic Visualisation for Endotracheal Intubation.** Supervisors: Prof.dr. M. Marcus / Prof.dr. A. van Zundert (University of Queensland).

Maria J. de Sousa Guerreiro: **The role of sensory modality in age-related distraction.** Supervisor: Prof.dr. C.M. van Heugten; Co-Supervisor: Dr. P.W.M. van Gerven.

Ine Rayen: **Effects of developmental fluoxetine exposure on neurobehavioral outcomes.** Supervisor: Prof.dr. H.W.M. Steinbusch; Co-Supervisors: Dr. J.L. Pawlusi / Dr. T.D. Charlier (Ohio University, USA).

Nynke M.G. Bodde: **Psychogenic non-epileptic seizures; a separate disorder or part of a continuum?** Supervisors: Prof.dr. R. van Oostenbrugge / Prof.dr. K. Vonck (UZ Gent); Co-Supervisors: Dr. R. Lazon / Dr. A. de Louw (Epilepsiecentrum Kempenhaeghe, Heeze).

Alejandro M. Gomez: **Novel strategies for making myasthenia less gravis: targeting plasma cells and the neuromuscular junction.** Supervisor: Prof.dr. M.H. De Baets; Co-Supervisors: Dr. M. Losen / Dr. P. Martinez-Martinez.

Mohammad S. Rahnama'i: **Prostaglandins and Phosphodiesterases in the Urinary Bladder Wall.** Supervisors: Prof.dr. Ph. Van Kerrebroeck / Prof.dr. S. de Wachter (Universiteit Antwerpen); Co-Supervisor: Dr. G. van Koeveeringe.

Mariken B. de Koning: **Studying biomarkers in populations at genetic and clinical high risk for psychosis.** Supervisors: Prof.dr. T. Amelsvoort / Prof.dr. J. Booij (AMC).

Fabien Boulle: **Epigenetic regulation of BDNF/TrkB signaling in the pathophysiology and treatment of mood disorders.** Supervisors: Prof.dr. H.W.M. Steinbusch / Prof.dr. L. Lanfumey (Universiteit Parijs); Co-Supervisors: Dr. D. van den Hove / Dr. G. Kenis.

2014

Iris Nowak-Maes: **Tinnitus; assessment of quality of life & cost-effectiveness.**

Supervisors: Prof.dr. M. Peters / Prof.dr. B. Kremer; Co-Supervisors: Dr. M. Joore / Dr. L. Anteunis.

Marjolein Huijts: **Cognitive function in patients with cerebral small vessel disease.** Supervisor: Prof.dr. R.J. van Oostenbrugge; Co-Supervisors: Dr. A.A. Duits / Dr. J. Staals.

Markus Gantert: **Fetal inflammatory injury as origin of long term disease: Lessons from animal models.** Supervisors: Prof.dr. B. Kramer / Prof.dr. L. Zimmermann; Co-Supervisor: Dr. A. Gavilanes.

Elke Kuypers: **Fetal development after antenatal exposures: Chorioamnionitis and maternal glucocorticoids.** Supervisors: Prof.dr. B.W. Kramer / Prof.dr. H.W. Steinbusch / Prof.dr. Suhas G. Kallapur (University of Cincinnati, Ohio, USA).

Pieter Kubben: **Ultra low-field strength intraoperative MRI for Glioblastoma Surgery.** Supervisor: Prof.dr. J.J. van Overbeek; Co-Supervisor: Dr. H. van Santbrink.

Laura Baijens: **Surface electrical stimulation of the neck for oropharyngeal dysphagia in Parkinson's disease: therapeutic aspects and reliability of measurement.** Supervisor: Prof.dr. B. Kremer; Co-Supervisor: Dr. R. Speyer, Townsville.

Janneke Hoeijmakers: **Small fiber neuropathy and sodium channels; a paradigm shift.** Supervisor: Prof.dr. R.J. van Oostenbrugge; Co-Supervisors: Dr. C.G. Faber / Dr. I.S.J. Merkies.

Stephanie Vos: **The Role of biomarkers in preclinical and prodromal Alzheimer's disease.** Supervisor: Prof.dr. F.R. Verhey; Co-Supervisor: Dr. P.J. Visser.

Muriel Doors: **The Value of Optical Coherence Tomography in Anterior Segment Surgery.** Supervisors: Prof.dr. R.M. Nuijts / Prof.dr. C.A. Webers; Co-Supervisor: Dr. T.T.J.M. Berendschot.

Anneke Maas: **Sleep problems in individuals with genetic disorders associated with intellectual disability.** Supervisors: Prof.dr. I. Curfs / Prof.dr. R. Didden.

Sebastiaan van Gorp: **Translational research on spinal cord injury and cell-based therapies; a focus on pain and sensorimotor disturbances.** Supervisors: Prof.dr. B. Joosten / Prof.dr. M. van Kleef; Co-Supervisors: Dr. J. Patijn / Dr. R. Deumens, KU Leuven.

Andrea Sannia: **High risk newborns and brain biochemical monitoring**. Supervisor: Prof.dr. J.S.H. Vles; Co-Supervisors: Dr. D. Gazzolo, Alessandria, Italy / Dr. A.W.D. Gavilanes.

Julie A.D.A. Dela Cruz: **Dopamine mechanisms in learning and memory: Evidence from rodent studies**. Supervisors: Prof.dr. H.W.M. Steinbusch / Prof.dr. R.J. Bodnar, New York; Co-Supervisor: Dr. B.P.F. Rutten.

René Besseling: **Brain wiring and neuronal dynamics; advances in MR imaging of focal epilepsy**. Supervisors: Prof.dr. A.P. Aldenkamp / Prof.dr.ir. W.H. Backes; Co-Supervisor: dr. J.F.A. Jansen.

Maria Quint-Fens: **Long-term care after stroke; development and evaluation of a long-term intervention in primary care**. Supervisors: Prof.dr. J.F.M. Metsemakers / Prof.dr. C.M. van Heugten / Prof.dr. M. Limburg, Almere; Co-Supervisor: dr. G.H.M.I. Beusmans.

Veronique Moulart: **Life after survival of a cardiac arrest; the heart of the matter**. Supervisors: Prof.dr. J.A. Verbunt / Prof.dr. C.M. van Heugten / Prof.dr. D.T. Wade, Oxford, UK.

Feikje Smeets: **The hallucinatory-delusional state: a crucial connection in the psychosis symptom network**. Supervisor: Prof.dr. J. van Os; Co-Supervisor: Dr. T. Lataster.

Lies Clerx: **Alzheimer's disease through the MR-eye; novel diagnostic markers and the road to clinical implementation**. Supervisor: Prof.dr. F. Verhey; Co-Supervisors: Dr. P.J. Visser / P. Aalten.

Sonny Tan: **The subthalamic nucleus in Parkinson's disease**. Supervisors: Prof.dr. Y. Temel / Prof.dr. H.W.M. Steinbusch / Prof.dr. T. Sharp, Oxford, UK / Prof.dr. V. Visser-Vandewalle, Koln.

Koen van Boxem: **The use of pulsed radiofrequency in the management of chronic lumbosacral radicular pain**. Supervisors: Prof.dr. M. van Kleef / Prof.dr. E.A.J. Joosten; Co-Supervisor: Assoc. Prof.dr. J. van Zundert.

Jérôme Waterval: **Hyperostosis cranialis interna**. Supervisors: Prof.dr. J.J. Manni / Prof.dr. R.J. Stokroos.

Sylvie Kolschoten-van der Kruijs: **Psychogenic non-epileptic seizures; the identification of neurophysiological correlates**. Supervisors: Prof.dr. A.P. Aldenkamp / Prof.dr. K.E.J. Vonck, Universiteit Gent; Co-Supervisors: Dr. J.F.A. Jansen / Dr. R.H.C. Lazon, Kempenhaeghe.

Wouter Pluijms: **Spinal cord stimulation and pain relief in painful diabetic: polyneuropathy, a translational approach**. Supervisors: Prof.dr. M. van Kleef / Prof.dr. E.A. Joosten; Co-supervisor: Dr. C.G. Faber.

Ron Handels: **Health technology assessment of diagnostic strategies for Alzheimer's disease**. Supervisors: Prof.dr. F.R.J. Verhey / Prof.dr. J.L. Severens (EUR); Co-Supervisor: Dr. M.A. Joore / Dr. C.A.G. Wolfs.

Evelyn Peelen: **Regulatory T cells in the pathogenesis of Multiple Sclerosis: potential targets for vitamin D therapy**. Supervisors: Prof.dr. R.M.M. Hupperts / Prof.dr. J.W. Cohen Tervaert; Co-Supervisor: Dr. J.G.M.C. Damoiseaux / Dr. M.M.G.L. Thewissen, Diepenbeek.

Reint Jellema: **Cell-based therapy for hypoxic-ischemic injury in the preterm brain**. Supervisors: Prof.dr. B.W.W. Kramer / Prof.dr. H.W.M. Steinbusch; Co-Supervisor: Dr. W.T.V. Germeraad / Dr. P. Andriessen, Veldhoven.

Maria Wertli: **Prognosis of Chronic Clinical Pain Conditions: The Example of Complex Regional Pain Syndrome 1 and Low Back Pain**. Supervisors: Prof.dr. M. van Kleef; Co-Supervisor: Dr. F. Brunner, Zürich / Dr. R. Perez, VUmc.

Dagmar Zeef: **An experimental model of Huntington's disease: Validation & Stimulation**. Supervisors: Prof.dr. Y. Temel / Prof.dr. H.W.M. Steinbusch; Co-supervisor: Dr. A. Jahanshahi.

Jeroen Decoster: **Breaking Down Schizophrenia into phenes, genes and environment**. Supervisors: Prof.dr. I. Myin-Germeys / Prof.dr. M. De Hert, KU Leuven; Co-Supervisor: Dr. R. van Winkel.

Eaja Anindya Sekhar Mukherjee: **Fetal Alcohol Spectrum Disorders: exploring prevention and management**. Supervisor: Prof.dr. L.M.G. Curfs; Co-Supervisor: Prof. S. Hollins, St. George's University of London, UK.

Catherine van Zelst: **Inside out; On stereotype awareness, childhood trauma and stigma in psychosis**. Supervisors: Prof.dr. Ph. Delespaul / Prof.dr. J. van Os.

Ibrahim Tolga Binbay: **Extended Psychosis Phenotype in the Wider Social Environment**. Supervisor: Prof.dr. J. van Os; Co-Supervisor: Dr. M. Drukker.

Frank Van Dael: **OCD matters in psychosis**. Supervisors: Prof.dr. J. van Os / Prof.dr. I. Myin-Germeys.

Pamela Kleikers: **NOXious oxidative stress: from head toe too and back**. Supervisors: Prof.dr. H.H.H.W. Schmidt / Prof.dr. H.W.M. Steinbusch; Co-Supervisor: Dr. B. Janssen.

José Luis Gerardo Nava: **In vitro assay systems in the development of therapeutic interventions strategies for neuroprotection and repair**. Supervisors: Prof.dr.med. J. Weis / Prof.dr. H.W.M. Steinbusch; Co-Supervisor: Dr. G.A. Brook, RWTH Aachen.

Eva Bollen: **Cyclic nucleotide signaling and plasticity**. Supervisors: Prof.dr. H.W.M. Steinbusch / Prof.dr. R. D'Hooge, KU Leuven; Co-Supervisor: Dr. J. Prickaerts.

2015

Jessica A. Hartmann: **A good laugh and a long sleep; Insights from prospective and ambulatory assessments about the importance of positive affect and sleep in mental health**. Supervisor: Prof.dr. J. van Os; Co-Supervisors: C.J.P. Simons / Dr. M. Wichers.

Bart Ament: **Frailty in old age; conceptualization and care innovations**. Supervisors: Prof.dr. G.I.J.M. Kempen / Prof.dr. F.R.J. Verhey; Co-Supervisor: Dr. M.E. de Vugt.

Mayke Janssens: **Exploring course and outcome across the psychosis-continuum**. Supervisor: Prof.dr. I. Myin-Germeys; Co-Supervisor: Dr. T. Lataster.

Dennis M.J. Hernau: **Dopayours is not dopamine: genetic, environmental and pathological variations in dopaminergic stress processing**. Supervisor: Prof.dr. I. Myin-Germeys; Co-Supervisors: Prof.dr. F.M. Mottaghy / Dr. D. Collip.

Ingrid M.H. Brands: **The adaptation process after acquired brain injury Pieces of the puzzle**. Supervisors: Prof.dr. C.M. van Heugten / Prof.dr. D.T. Wade, Oxford UK; Co-Supervisors: Dr. S.Z. Stapert / Dr. S. Köhler.

Francesco Risso: **Urinary and salivary S100B monitoring in high risk infants**. Supervisor: Prof.dr. J.S.H. Vles; Co-Supervisors: Dr. D. Gazzolo, Genoa, Italy / Dr. A.W.D. Gavilanes.

Alessandro Borghesi: **Stem and Progenitor Cells in Preterm Infants: Role in the Pathogenesis and Potential for Therapy**. Supervisor: Prof.dr. L. Zimmermann; Prof.dr. B. Kramer; Co-Supervisors: Dr. D. Gazzolo, Genoa, Italy / Dr. A.W.D. Gavilanes.

Claudia Menne-Lothmann: **Affect dynamics; A focus on genes, stress, and an opportunity for change**. Supervisor: Prof.dr. J. van Os; Co-Supervisors: Dr. M. Wichers / Dr. N. Jacobs.

Martine van Nierop: **Surviving childhood new perspectives on the link between childhood trauma and psychosis**. Supervisors: Prof.dr. I. Myin-Germeys / Prof.dr. J. van Os; Co-Supervisor: Dr. R. van Winkel.

Sylvia Klinkenberg: **VNS in children; more than just seizure reduction**. Supervisors: Prof.dr. J. Vles / Prof.dr. A. Aldenkamp; Co-Supervisor: Dr. H. Majoie.

Anouk Linssen: **Considerations in designing an adult hearing screening programme**. Supervisor: Prof.dr. B. Kremer; Co-Supervisors: Dr. L. Anteunis / Dr. M. Joore.

Janny Hof: **Hearing loss in young children; challenges in assessment and intervention**. Supervisors: Prof.dr. B. Kremer / Prof.dr. R. Stokroos / Prof.dr. P. van Dijk, RUG; Co-Supervisor: Dr. L. Antheunis.

Kimberly Cox-Limpens: **Mechanisms of endogenous brain protection; Clues from the transcriptome**. Supervisors: Prof.dr. J. Vles / Prof.dr. L. Zimmermann; Co-Supervisor: Dr. A. Gavilanes.

Els Vanhoutte: **Peripheral Neuropathy outcome measures; Standardisation (PeriNomS) study part 2: Getting consensus**. Supervisors: Prof.dr. C. Faber / Prof.dr. P. van Doorn; Co-Supervisor: Dr. I. Merkies, Spaarne ziekenhuis Hoofddorp.

Mayienne Bakkers: **Small fibers, big troubles; diagnosis and implications of small fiber neuropathy**. Supervisors: Prof.dr. C. Faber / Prof.dr. M. de Baets; Co-Supervisor: Dr. I. Merkies, Spaarne ziekenhuis Hoofddorp.

Ingrid Kramer: **Zooming into the micro-level of experience: An approach for understanding and treating psychopathology.** Supervisor: Prof.dr. J. van Os; Co-Supervisors: Dr. M. Wichers, UMC Groningen / Dr. C. Simons.

Esther Bouman: **Risks and Benefits of Regional Anesthesia in the Perioperative Setting.** Supervisors: Prof.dr. M. van Kleef / Prof.dr. M. Marcus, HMC, Qatar / Prof.dr. E. Joosten; Co-Supervisor: Dr. H. Gramke.

Mark Janssen: **Selective stimulation of the subthalamic nucleus in Parkinson's disease; dream or near future.** Supervisors: Prof.dr. Y. Temel / Prof.dr. V. Visser-Vandewalle, Keulen / Prof.dr. A. Benazzouz, Bordeaux, France.

Reina de Kinderen: **Health Technology Assessment in Epilepsy; economic evaluations and preference studies.** Supervisors: Prof.dr. S. Evers / Prof.dr. A. Aldenkamp; Co-Supervisor: Dr. H. Majoie / Dr. D. Postulat, GGZ O-Brabant.

Saskia Ebus: **Interictal epileptiform activity as a marker for clinical outcome.** Supervisors: Prof.dr. A. Aldenkamp / Prof.dr. J. Arends, TUE / Prof.dr. P. Boon, Universiteit Gent, België.

Inge Knuts: **Experimental and clinical studies into determinants of panic severity.** Supervisor: Prof.dr. I. Myin-Germeys; Co-Supervisor: Dr. K. Schruers; Influencing panic.

Nienke Tielemans: **Proactive coping post stroke: The Restored4Stroke Self-Management study.** Supervisors: Prof.dr. C. van Heugten / Prof.dr. J. Visser-Meily, UMC Utrecht; Co-Supervisor: Dr. V. Schepers, UMC Utrecht.

Tom van Zundert: **Improvements Towards Safer Extraglottic Airway Devices.** Supervisors: Prof.dr. A.E.M. Marcus / Prof.dr. W. Buhre / Prof.dr. J.R. Brimacombe, Queensland, Australia / Prof.dr. C.A. Hagberg.

Tijmen van Assen: **Anterior Cutaneous Nerve Entrapment Syndrome Epidemiology and surgical management.** Supervisors: Prof.dr. G.L. Beets / Prof.dr. M. van Kleef / Dr. R.M.H. Roumen / Dr. M.R.M. Scheltinga, MMC Veldhoven.

Rohit Shetty: **Understanding the Clinical, Immunological and Genetic Molecular Mechanisms of Keratoconus.** Supervisors: Prof.dr. R.M.M.A. Nuijts / Prof.dr. C.A.B. Webers.

Christine van der Leeuw: **Blood, bones and brains; peripheral biological endophenotypes and their structural cerebral correlates in psychotic disorder.** Supervisor: Prof.dr. J. van Os; Co-supervisor: Dr. M. Marcelis.

Sanne Peeters: **The Idle Mind Never Rests; functional brain connectivity across the psychosis continuum.** Supervisor: Prof.dr. J. van Os; Co-supervisor: dr. M. Marcelis.

Nick van Goethem: **$\alpha 7$ nicotinic acetylcholine receptors and memory processes: mechanistic and behavioral studies.** Supervisor: Prof.dr. H.W.M. Steinbusch; Co-supervisor: Dr. J. Prickaerts.

Nicole Leibold: **A Breath of fear; a translational approach into the mechanisms of panic.** Supervisor: Prof.dr. H.W.M. Steinbusch; Co-supervisors: Dr. K.R.J. Schruers / Dr. D.L.A. van den Hove.

Renske Hamel: **The course of mild cognitive impairment and the role of comorbidity.** Supervisor: Prof.dr. F.R.J. Verhey; Co-supervisors: Dr. I.H.G.B. Ramakers / Dr. P.J. Visser.

Lucia Speth: **Effects of botulinum toxin A injections and bimanual task-oriented therapy on hand functions and bimanual activities in unilateral Cerebral Palsy.** Supervisors: Prof.dr. J. Vles; Prof.dr. R. Smeets; Co-supervisor: Dr. Y. Janssen-Potten, Adelante Hoensbroek.

Yuan Tian: **The effects of Lutein on the inflammatory pathways in age-related macular degeneration (AMD).** Supervisors: Prof.dr. C. Webers; Prof.dr. A. Kijlstra, WUR; Co-supervisor: Dr. M. Spreeuwenberg; Dr. H. Tange.

Peggy Spauwen: **Cognition and Type 2 diabetes; the interplay of risk factors.** Supervisors: Prof.dr. F. Verhey; Prof.dr. C. Stehouwer; Co-supervisor: Dr. M. van Boxtel

Marc Hilhorst: **Crescentic glomerulonephritis in ANCA associated vasculitis.** Supervisors: Prof.dr. J. Cohen-Tervaert; Co-supervisor: Dr. P. van Paassen

Martin Gevonden: **The odd one out: exploring the nature of the association between minority status and psychosis.** Supervisors: Prof.dr. J-P. Selten; Prof.dr. J. Booij, Uva; Prof.dr. I. Myin-Germeys

Bart Bialosterski: **Structural and functional aspects of sensory-motor Interaction in the urinary bladder.** Supervisors: Prof.dr. Ph. Van Kerrebroeck; Prof.dr. S. De Wachter, UvAntwerpen; Co-supervisors: Dr. G. van Koeveringe; Dr. M. Rahnama'i.

Alexandra König: **The use of information and communication technologies (ICT) for the assessment of patients with Alzheimer's Disease and related disorders.** Supervisors: prof.dr. F. Verhey; prof.dr. Ph. Robert, Nice, Fr; Co-supervisors: dr. P. Aalten; dr. R. David, Nice, Fr.

Micheline Chenault: **Assessing Readiness for Hearing Rehabilitation.** Supervisors: prof.dr. M.P.F. Berger; prof.dr. B. Kremer; Co-supervisor: dr. L.J.C. Anteunis.

Anand Vinekar: **Retinopathy of Prematurity. Recent advances in tele-medicine screening, risk factors and spectral domain optical coherence tomography imaging.** Supervisor: prof.dr. C.A.B. Webers; Co-supervisor: dr. N.J. Bauer

Fleur van Dooren: **Diabetes and Depression: exploring the Interface between Pathophysiological and Psychological factors.** Supervisors: prof.dr. F.R.J. Verhey; prof.dr. J.K.L. Denollet, UvT; prof.dr. F. Pouwer, UvT; Co-supervisor: dr. M.T. Schram.

Gabriella Pons van Dijk: **Taekwondo and physical fitness components in middle-aged healthy volunteers; the Sekwondo study.** Supervisors: prof.dr. J. Lodder; prof.dr. H. Kingma; Co-supervisor: dr. A.F. Lenssen.

Yara Pujol López: **Development and psychoneuroimmunological mechanisms in depression.** Supervisor: prof.dr. H.W.M. Steinbusch; Co-supervisors: Dr. G. Kenis; Dr. D. van den Hove; Dr. Aye Mu Myint, München.

Romina Gentier: **UBB⁺¹; an important switch in the onset of Alzheimer's disease.** Supervisors: Prof. H. Steinbusch; Prof. D. Hopkins; Co-supervisor: Dr. F. van Leeuwen.

Sanne Smeets: **Insights into insight: studies on awareness of deficits after acquired brain injury.** Supervisor: Prof. C. van Heugten; Prof. R. Ponds; Co-supervisor: Dr. I. Winkens

Kim Beerhorst: **Bone disease in chronic epilepsy: fit for a fracture.** Supervisor: Prof. A. Aldenkamp; Prof. R. van Oostenbrugge; Co-supervisor: Dr. P. Verschuure.

Alex Zwanenburg: **Cerebral and cardiac signal monitoring in fetal sheep with hypoxic- ischemic encephalopathy.** Supervisor: Prof. T. Delhaas; Prof. B. Kramer; Co-supervisors: Dr. T. Wolfs; Dr. P. Andriessen, MMC.

Ismail Sinan Guloksuz: **Biological mechanisms of environmental stressors in psychiatry.** Supervisor: Prof. J. van Os; Co-supervisors: Dr. B. Rutten; Dr. M. Drukker.

Seyed Ehsan Pishva MD: **Environmental Epigenetics in mental health and illness.** Supervisor: Prof.dr. J. van Os; Co-supervisors: Dr. B.P.F. Rutten; Dr. G. Kenis.

Ankie Hamaekers: **Rescue ventilation using expiratory ventilation assistance; innovating while clutching at straws.** Supervisors: Prof.dr. W.F. Buhre; Prof.dr. M. van Kleef.

Rens Evers. **22q11.2 deletion syndrome: intelligence, psychopathology and neurochemistry at adult age.** Supervisors: Prof.dr. L.M.G. Curfs; Prof.dr. T. v. Amelsvoort.

Sarah-Anna Heschem. **Novel insights towards memory restoration.** Supervisor: Prof.dr. Y. Temel; Co-supervisor: Dr. A. Blokland; Dr. A. Jahanshahi.

João P. da Costa Alvares Viegas Nunes. **Insulin receptor sensitization improves affective pathology in various mouse models.** Supervisor: Prof.dr. H.W.M. Steinbusch; Co-supervisors: Dr. K-P. Lesch; Dr. T. Strekalova; Dr.B.H. Cline, Oxford.

Yanny Ying-Yee Cheng. **Clinical Outcomes After Innovative Lamellar Corneal Transplantation Surgery.** Supervisor: Prof.dr. R.M.M.A. Nuijts; Co-supervisor: Dr. J.S.A.G. Schouten.

2016

Oliver Gerlach. **Parkinson's disease, deterioration during hospitalization.** Supervisor: Prof.dr. R. van Oostenbrugge; Co-supervisor: Dr. W. Weber.

Remo Arts. **Intracochlear electrical stimulation to suppress tinnitus.** Supervisor: Prof.dr. R.J. Stokroos; Co-supervisor: Dr. E.L.J. Georg.

Mitchel van Eeden. **The €- Restore4stroke study: Economic evaluation of stroke care in the Netherlands.** Supervisors: Prof.dr.mr. S.M.A.A. Evers; Prof.dr. C.M. v. Heugten; Co-supervisor: dr. G.A.P. van Mastrigt.

Pim Klarenbeek. **Blood pressure and cerebral small vessel disease.** Supervisor: Prof.dr. R.J. van Oostenbrugge; Co-supervisor: Dr. J. Staals.

Ramona Hohnen. **Peripheral pharmacological targets to modify bladder contractility.** Supervisor: Prof.dr. Ph.E.V. van Kerrebroeck; Co-supervisors: Dr. G.A. van Koevinge; Dr. M.A. Sahnma; Dr. C. Meriaux.

Ersay Kocabicak. **Deep brain stimulation of the subthalamic nucleus: Clinical and scientific aspects.** Supervisors: Prof.dr. Y. Temel; Prof.dr. K. van Overbeeke; Co-supervisor: Dr. A. Jahanshahi.

Sven Akkerman. **Temporal aspects of cyclic messenger signaling in object recognition memory; a pharmacological approach.** Supervisor: Prof.dr. H.W.M. Steinbusch; Co-supervisors: dr. J. Prickaerts; dr. A. Blokland.

Anja Moonen. **Emotion and Cognition in Parkinson's disease; etiology and neurobiological mechanisms.** Supervisor: Prof.dr. F.R.J. Verhey; Co-supervisor: dr. A.F.G. Leentjens.

Anna Schüth. **Three-dimensional bladder tissue morphology.** Supervisors: Prof.dr. G.A. van Koevinge; Prof.dr. M. v. Zandvoort, Aachen; Prof.dr. Ph. V. Kerrebroeck.

Elisabeth van der Ven. **Ethnic minority position as risk indicator for autism- Spectrum and psychotic disorders.** Supervisors: Prof.dr. J.P. Selten; Prof.dr. J. van Os.

Zuzana Kasanova. **Environmental reactivity for better or worse; The impact of stress and reward on neurochemistry, affect and behavior across the psychosis continuum.** Supervisor: Prof.dr. I. Myin-Germeys, KU Leuven/UM; Co-supervisor: dr. D. Collip.

Danielle Lambrechts. **Ketogenic diet therapies; treatment for children and adults with refractory epilepsy.** Supervisors: Prof.dr. H.J.M. Majoie; Prof.dr. J.S.H. Vles; Prof.dr. A.P. Aldenkamp; Co-supervisor: dr. A.J.A. de Louw, Kempenhaeghe, Heeze.

Frank van Bussel. **Advanced MRI in diabetes; cerebral biomarkers of cognitive decrements.** Supervisors: Prof.dr.ir. W.H. Backes; Prof.dr. P.A.M. Hofman; Co-supervisor: dr. J.F.A. Jansen.

Lisa Schönfeldt. **Neurostimulation to treat brain injury?** Supervisors: Prof.dr. Y. Temel; Prof.dr. S. Hendriks, Hasselt; Co-supervisor: dr. A. Jahanshahi.

Rianne Geerlings. **Transition in patients with childhood-onset epilepsy; a long way to adulthood.** Supervisor: Prof.dr. A.P. Aldenkamp; Co-supervisors: dr. A.J.A. de Louw, dr. L.M.C. Gottmer, Kempenhaeghe.

Nele Claes. **B cells as multifactorial players in multiple sclerosis pathogenesis: insights from therapeutics.** Supervisors: Prof.dr. V. Somers, Hasselt; Prof.dr. R. Hupperts; Co-supervisors: Prof.dr. P. Stinissen, dr. J. Fraussen, Hasselt.

Olaf Schijns. **Epilepsy surgery and biomarkers from history to molecular imaging.** Supervisors: Prof.dr. J.J. van Overbeeke; Prof.dr. H. Clustermann, Aachen; Co-supervisors: dr. G. Hoogland; dr. M.J.P. v. Kroonenburgh.

Lizzy Boots. **Balanced and Prepared; development and evaluation of a supportive e- health intervention for caregivers of people with early-stage dementia.** Supervisors: Prof.dr. F.R.J. Verhey; Prof.dr. G.I.J.M. Kempen; Co-supervisor: dr. M.E. de Vugt.

Wouter Donders. **Towards patient-specific (cerebro-) vascular model applications.** Supervisors: Prof.dr. T. Delhaas; Prof.dr.ir. F.N. van de Vosse, TUE; Co-supervisor: dr.ir. W. Huberts.

Sizzle Vanterpool. **The implications of intrauterine invasion by microbes for placental Pathology and the occurrence of adverse pregnancy outcomes.** Supervisor: Prof.dr. B.W. Kramer. Co-supervisors: dr. J.V. Been, Erasmus MC Rotterdam, dr. U von Rango.

Manuela Heins. **The Relationship between Social Adversity, Psychosis, and Depression across an Individual's Life Span.** Supervisor: Prof.dr. I. Myin-Germeys.

Christianus van Ganzewinkel. **NEONATAL PAIN; Out of Sight, Out of Mind?** Supervisor: Prof.dr. B.W.W. Kramer; Co-supervisor: dr. P. Andriessen, MMC Veldhoven.

Anne-Hilde Muris. **Hype or hope? Vitamin D in multiple sclerosis; A clinical and immunological perspective.** Supervisor: Prof.dr. R.M.M. Hupperts; Co-supervisor: dr. J.G.M.C. Damoiseaux.

Gerard Bode. **The link between ceramide transporters, innate Immunity and Alzheimer's disease.** Supervisor: Prof.dr. M.H.V. de Baets; Co-supervisors: dr. P. Martinez, dr. M. Losen.

Jo Stevens. **Advanced diagnostics and therapeutics for Alzheimer's disease.** Supervisor: Prof.dr. M. de Baets; Co-supervisors: dr. M. Losen, dr. P. Martinez-Martinez.

Rosan Luijckx. **Stress and pain in muscles and brain; developing psychophysiological paradigms to examine stress and pain interactions.** Supervisors: Prof.dr. J.J. van Os; Prof.dr.ir. H.J. Hermens, UT; Co-supervisor: dr. R. Lousberg.

M.C. Haanschoten. **Towards efficient cardiac surgery – the integrating role of anesthesiology and intensive care.** Supervisors: Prof. dr. W. Buhre; Prof. dr. A. van Zundert (Queensland); Co-supervisors: Dr. M.A. Soliman Hamad; Dr. A. van Straten (Catharina zkhs).

Harmen Jan van de Haar. **Microvascular and blood-brain barrier dysfunction in Alzheimer's disease.** Supervisor: Prof.dr.ir. W. Backes; Prof.dr. F. Verhey; Co-supervisor: Dr. J. Jansen; Dr.ir. M. v. Osch, LUMC.

Coenraad Itz. **Chronic low back pain, considerations about: Natural Course, Diagnosis, Interventional Treatment and Costs.** Supervisor: Prof.dr. M. van Kleef; Prof.dr. F. Huygen, EUR; Co- supervisor: Dr. B. Ramackers.

Willemijn Jansen. **The Path of Alzheimer's disease: from neuropathology to clinic.** Supervisor: Prof.dr. F. Verhey; Co-supervisors: Dr. P.J. Visser; Dr. I. Ramakers.

Ligia dos Santos Mendes Lemes Soares. **Phosphodiesterase inhibitors: a potential therapeutic approach for ischemic cerebral injury.** Supervisor: Prof.dr. H.W.M. Steinbusch; Co-supervisors: Dr. R.M. Weffort de Oliveira, Brazil; Dr. J. Prickaerts

Martijn Broen. **Anxiety and depression in Parkinson's disease.** Supervisor: Prof.dr. R.J. van Oostenbrugge; Co-supervisors: Dr. A.F.G. Leentjens; Dr. M.L. Kuijf.

Sandra Schipper. **Extrasynaptic receptors as a treatment target in epilepsy.** Supervisor: Prof.dr. J.H.S. Vles; Co-supervisors: Dr. G. Hoogland; Dr. S. Klinkenberg; Dr. M.W. Aalbers, RUG.

João Casaca Carreira. **Making sense of Antisense Oligonucleotides Therapy in Experimental Huntington's disease.** Supervisor: Prof.dr. Y. Temel; Co-supervisors: Dr. A. Jahanshahi; Dr. W. van Roon-Mom, LUMC.

Dominique IJff. **Trick or Treat? Cognitive side-effects of antiepileptic treatment.** Supervisors: Prof.dr. A.P. Aldenkamp; Prof.dr. M. Majoie; Co-supervisors: Dr. J. Jansen; Dr. R. Lazon, Kempenhaeghe.

Alfredo Ramirez. **Neurogenetic approach in neurodegenerative disorders.** Supervisors: Prof.dr. B.P.F. Rutten; Prof.dr. H.W.M. Steinbusch; Prof.dr. M.M. Nöthen, University of Bonn.

Nienke Visser. **Toric Intraocular lenses in cataract surgery.** Supervisor: Prof.dr. R.M.M.A. Nuijts; Co-supervisor: Dr. N.J.C. Bauer.

Jakob Burgstaller. **Prognostic indicators for patients with degenerative lumbar spinal stenosis.** Supervisor: Prof.dr. M. van Kleef; Co-supervisors: Dr. M.M. Wertli, University of Zurich; Dr. H.F. Gramke.

Mark van den Hurk. **Neuronal Identity and Maturation: Insights from the Single-Cell Transcriptome.** Supervisors: Prof.dr. H.W.M. Steinbusch; Prof.dr. B.P.F. Rutten; Co- supervisors: Dr. G. Kenis; Dr. C. Bardy, Adelaide.

Maria Nikiforou. **Prenatal stress and the fetal gut. Potential interventions to prevent adverse outcomes.** Supervisors: Prof.dr. B.W. Kramer; Prof.dr. H.W. Steinbusch; Co- supervisor: Dr. T.G. Wolfs.

Janneke Peijnenborgh. **Assessment of cognition, time perception, and motivation in children.** Supervisors: Prof.dr. J.S.H. Vles; Prof.dr. A.P. Aldenkamp; Co-supervisors: Dr. J. Hendriksen; Dr. P. Hurks.

Joany Millenaar. **Young onset dementia; towards a better understanding of care needs and experiences.** Supervisors: Prof.dr. F. Verhey; Prof.dr. R. Koopmans, RUN; Co-supervisors: Dr. M. de Vugt; Dr. C. Bakker, RUN.

2017

Adriana Smits. **Perinatal factors and hearing outcome.** Supervisors: Prof.dr. R.J. Stokroos; Prof.dr. B.W. Kramer; Prof.dr. B. Kremer.

Angela Bouwmans. **Transcranial sonography in parkinsonian disorders: clear window or blurred vision.** Supervisor: Prof.dr. W.H. Mess; Co-promotors: Dr. W.E.J. Weber; Dr. A.F.G. Leentjens.

Björn K. Stessel. **Patient centred care after day surgery: scope for improvement.** Supervisors: Prof.dr. W. Buhre; Prof.dr. B. Joosten. Co-supervisor: Dr. A.H. Gramke.

Jan Guy Bogaarts. **Quantitative EEG and machine learning methods for the detection of epileptic seizures and cerebral asymmetry.** Supervisor: Prof.dr. W.M. Mess; Co-supervisor: Dr.ir. J.P.H. Reulen; Dr.ir. E.D. Gommer.

Martin M. Müller. **Pregnancy derived products for treatment of perinatal brain injuries.** Supervisors: Prof.dr. B.W.W. Kramer; Prof.dr. D. Surbek, Bern; Co-supervisors: Dr. T. Wolfs; Dr. G. Gavilanes.

Daan Ophelders. **Novel treatment strategies for the protection of the preterm brain; Re- balancing inflammation and regeneration.** Supervisor: Prof.dr. B. Kramer; Co-supervisor: Dr. T. Wolfs; Dr. R. Jellema.

Rosalie van Knippenberg. **Experience sampling in dementia care; an innovative intervention to support caregivers in daily life.** Supervisors: Prof.dr. F. Verhey; Prof.dr. R. Ponds; Prof.dr. I. Myin-Germeys, KU Leuven; Co-supervisor: Dr. M. de Vugt.

Claudia Vingerhoets. **Investigating neurobiological mechanisms underlying comorbid cognitive symptoms in psychosis and substance use.** Supervisors: Prof.dr. T. van Amelsvoort; Prof.dr. J. Booij, UvA; Co-supervisor: Dr. O. Bloemen

Dennis Oerlemans. **Evolution of Neuromodulation for Lower Urinary Tract Dysfunction; Past, Present and Future.** Supervisors: Prof.dr. Ph. van Kerrebroeck; Prof.dr. G. van Koeveing. Co-supervisors: Dr. E. Weil; Dr. T. Marcelissen.

Marion Levy. **Evaluation of BDNF/TrkB signaling as a common target in the treatment of major depression and Alzheimer's disease.** Supervisors: Prof.dr. H. Steinbusch; Prof. L. Lanfumey, Université Paris Descartes, France. Co-supervisors: Dr. G. Kenis; Dr. D. van den Hove.

Patrick Domen. **Stay connected: a family-based diffusion imaging study in psychotic disorder.** Supervisor: Prof.dr. J. van Os. Co-supervisor: Dr. M. Marcelis

Geor Bakker. **Innovative Approaches to Understanding the Neurobiology of Psychosis.** Supervisors: Prof.dr. T. van Amelsvoort; Prof.dr. J. Booij, UvA. Co-supervisor: dr. M. Caan, UvA; dr. O. Bloemen.

Wilma Boevink. **HEE! Over Herstel, Empowerment en Ervaringsdeskundigheid in de psychiatrie.** Supervisors: Prof.dr. J. van Os; Prof.dr. Ph. Delespaul. Co-supervisor: dr. H. Kroon.

Natalia Markova . **Modified swim test as a mouse depression paradigm of enhanced Cognitive processing: the role of GSK3β.** Supervisor: Prof.dr. H. Steinbusch; Prof.dr. K-P. Lesch, University of Wuerzburg. Co-supervisor: Dr. T. Strelakova.

Merijn van de Laar. **Individual differences in insomnia; implications of Psychological factors for diagnosis and treatment.** Supervisor: Prof.dr. A. Aldenkamp; Prof.dr. D. Pevernagie, Universiteit Gent. Co-supervisor: Dr. S. Overeem, TUE.

Willem Buskermolen. **If only I could tell ...; Measuring predictors for challenging behaviour in people with both intellectual disability and hearing impairment.** Supervisor: Prof.dr. A. Aldenkamp. Co-supervisor: Dr. J. Hoekman, UL.

Kay Deckers. **The role of lifestyle factors in primary prevention of dementia; an epidemiological perspective.** Supervisor: Prof.dr. F. Verhey. Co-supervisor: Dr. M. van Bortel; Dr. S. Köhler.

Brechje Dandachi-FitzGerald. **Symptom validity in clinical assessments.** Supervisors: Prof.dr. R. Ponds; Prof.dr. F. Verhey.

Maurice Theunissen. **Understanding factors affecting postoperative Quality of Life.** Supervisors: Prof.dr. M. Peters, Prof.dr. M. Marcus. Co-supervisor: Dr. H. Gramke.

Anna Cleutjens. **COgnitive-Pulmonary Disease? Neuropsychological functioning in patients with COPD.** Supervisors: Prof.dr. E. Wouters, Prof.dr. R. Ponds. Co-supervisors: Dr. D. Janssen, Horn, Dr. J. Dijkstra.

Laura Serpero. **Next Generaton Biomarkers in Perinatal Medicine: S100B Protein.** Supervisors: Prof.dr. D. Gazzalo, Alessandria, Italy; Prof.dr. B.W.W. Kramer. Co-supervisor: Dr. A.W.D. Gavilanes.

Alessandro Varrica. **S100B Protein and Congenital Heart Diseases: Brain Aspects.** Supervisors: Prof.dr. D. Gazzalo, Alessandria, Italy; Prof.dr. J.S.H. Vles; Prof.dr. L.J.I. Zimmermann. Co-supervisor: Dr. A.W.D. Gavilanes.

Pim R.A. Heckman. **Targeting phosphodiesterase type 4 for improving cognitive fronto- striatal function: a translational approach.** Supervisor: Prof.dr. J.G. Ramaekers. Co- supervisors: Dr. J.H.H.J.. Prickaerts; Dr. A. Blokland.

Sven van Poucke. **Platelets, form sample to big data; exploring granularity in platelet research.** Supervisors: Prof.dr. M.A.E. Marcus; Prof.dr. W. Buhre. Co-supervisor: Dr. M. Lancé.

Désirée M.J. Vrijens. **Dysfunctions of the Lower Urinary Tract and Affective Symptoms.** Supervisors: Prof.dr. Ph.E.V. van Kerrebroeck; Prof.dr. G.A. van Koevinge. Co- supervisors: Dr. C. Leue.

Tamar van Veenendaal. **Neurotransmitters & Networks. An MR view on epilepsy and antiepileptic drugs.** Supervisors: Prof.dr.ir. W.H. Backes; Prof.dr. A.P. Aldenkamp. Co- supervisor: Dr. J.F.A. Jansen.

Evelien M. Barendse. **Autism Spectrum Disorders in High functioning Adolescents; Diagnostic considerations (AHA).** Supervisors: Prof.dr. A.P. Aldenkamp; Prof.dr. R.P.C. Kessels, Radboud University.

Roy Lardenoije. **A venture into the epigenetics of aging and Alzheimer's Disease.** Supervisors: Prof.dr. B.P.F. Rutten; Prof.dr. H.W.M. Steinbusch. Co-supervisors: Dr. D. van den Hove; Dr. C.A. Lemere, USA.

Charlotte L. Mentzel. **The course recognition and treatment of movement disorders in severe mental illness.** Supervisors: Prof.dr. P.N. van Harten; Prof.dr. M.A.J. de Koning- Tijssen, UMCG. Co-supervisor: Dr. P.R. Bakker.

Tim Batink. **Third Wave Behaviour Therapy: Process Measures and Contextual Interventions.** Supervisors: Prof.dr. F.P.M.L. Peeters; Prof.dr. J.J. van Os; Prof.dr. M.C. Wichers, UMC Groningen.

Kevin L.J. Rademakers. **Detrusor Underactivity: From Theory to Clinical Assessment.** Supervisors: Prof.dr. G.A. van Koevinge; Prof.dr. Ph.E.V. van Kerrebroeck. Co-supervisor: Dr. M. Oelke.

Iris M.J. Lange. **Should I stay or should I go ? Brain mechanisms underlying fear and safety learning, and exposure therapy outcome.** Supervisors: Prof.dr. K.R.J. Schruers; Prof.dr. T.A.M.J. van Amelsfoort. Co-supervisor: Dr. L. Goossens.

Ruben G.F. Hendriksen. **Evidence for a dystrophin-associated encephalopathy in Duchenne Muscular Dystrophy.** Supervisor: Prof.dr. J.S.H. Vles. Co-supervisors: Dr. G. Hoogland; Dr. M.W. Aalbers, UMC Groningen.

Michael Gofeld. **Strengths and limitations of the lumbar spine ultrasound-guided interventions.** Supervisor: Prof.dr. M. van Kleef. Co-supervisor: Dr. M. Sommer.

Willem A.R. Zwaans. **Strategies for chronic inguinal pain.** Supervisor: Prof.dr. M. van Kleef. Co-supervisors: Dr. R.H.M. Roumen; Dr. M.R.M. Scheltinga, MMC Veldhoven.

Linda M. Rolf. **Mapping the effects of vitamin D in multiple sclerosis A 3D Perspective.** Supervisor: Prof.dr. R.M.M. Hupperts. Co-supervisors: Dr. J.G.M.C. Damoiseaux; Dr. J.J.F.M. Smolders, CWZ Nijmegen.

Maarten van Beek. **Spinal Cord Stimulation in Clinical and Experimental Painful Diabetic Polyneuropathy.** Supervisors: Prof.dr. E.A. Joosten; Prof.dr. M. van Kleef. Co- supervisor: Dr. S.M.J. van Kuijk.

Melina Barkhuizen. **Genetic and perinatal risk factors for movement disorders.** Supervisors: prof.dr. B.W.W. Kramer, prof.dr. H.W.M. Steinbusch, Prof.dr. A.F. Grobler. Co- supervisor: dr. A.W.D.Gavilanes-Jimenez.

Renske Uiterwijk. **Cognitive function and cerebral small vessel disease in hypertension.** Supervisor: prof.dr. R.J. van Oostenbrugge. Co-supervisor: Dr. J.E.A. Staals.

Elles Douven. **Depression and apathy after stroke.** Supervisor: prof.dr. F.R.J. Verhey. Co-supervisors: Dr. P. Aalten, dr. J. Staals.

Mauro Pessia. **Brain K⁺ Channels: from molecular and physiological features to autism spectrum disorder and intellectual disability.** Supervisors: prof.dr. H.W.M. Steinbusch, prof.dr. M.B. Donati, It.

Carsten Leue. **Hyperarousal in the Hospital and what to do about it: the MED-PSYCH- NET - a transitional network approach fostering personalized care in psychosomatic medicine.** Supervisors: Prof.dr. J. van Os, Prof.dr. A. Masclee. Co-supervisors: Dr. J. Strik, Dr. J. Kruimel

Andrea S. Herrera Soto. **Aminochrome, an endotoxin for inducing a new rat model of Parkinson's Disease.** Supervisor: prof.dr. H.W.M. Steinbusch. Co-supervisors: Prof.dr. Juan Segura-Aguilar; prof. G. Diaz-Veliz, Santiago of Chile

Eline E.B. de Clerck. **Ocular neurodegenerative changes and macular cysts in prediabetes and type 2 diabetes.** Supervisors: Prof.dr. C.A.B. Webers, Prof.dr. C.D.A. Stehouwer. Co-supervisor: Dr. J.S.A.G. Schouten

Steven T.H. Honings. **Exploring psychosis and multidirectional violence: a prospective study in the general population.** Supervisor: Prof.dr. J. van Os. Co-supervisor: Dr. M. Drukker

2018

Sau May Wong. **Advances in Microvasculair MRI Techniques: Breaking the Pathophysiological Barriers in Cerebral Small Vessel Disease.** Supervisor: Prof.dr. W.H. Backes, Prof.dr. R.J. van Oostenbrugge. Co-supervisor: Dr. J.F.A. Jansen

Mark B.N. van Winkel. **Lonely at heart and stressed in company of Others; the influence of daily life social experiences and emotions on depression.** Supervisors: prof.dr. F. Peeters; prof.dr. I. Myin-Germeys, KU Leuven/UM; prof.dr. M. Wichers, UMC Groningen

Harsha Birur Laxmana Rao. **Revisiting the vascular theory of glaucoma using optical coherence tomography angiography.** Supervisors: prof.dr. C.A.B. Webers; prof.dr. R.N. Weinreb, University of California, San Diego

Babette L.R. Reijis. **Cognitive correlates of cerebrospinal fluid biomarkers for Alzheimer's disease.** Supervisor: prof.dr. F.R.J. Verhey. Co-supervisors: Dr. P.J. Visser; dr. I.H.G.B. Ramakers

Rachel Slangen. **Spinal cord stimulation in painful diabetic peripheral Neuropathy. Clinical- and cost-effectiveness.** Supervisors: prof.dr. M. van Kleef; Prof.dr. C. Dirksen; prof.dr. C. Faber

Ganne Chaitanya. **Epilepsy: A network disorder.** Supervisors: prof.dr. A.P. Aldenkamp; prof. P. Satishchandra, NIMHANS, Bangalore, India. Co-supervisors: Dr. J.F.A. Jansen; Dr. S. Zinger, TUE

Sumitha Rajendrarao. **New Insight into the Multifaceted Pathogenic Mechanisms of Sporadic Amyotrophic Lateral Sclerosis.** Supervisors: prof.dr. B.W. Kamer; prof.dr. H.W. Steinbusch. Co-supervisor: prof. T.R. Raju, NIMHANS, Bangalore, India

Suzanne Roggeveen. **Interference of mobile phone with electrophysiology and emotions; results from short-term experimental studies.** Supervisor: Prof.dr. J. van Os. Co-supervisor: Dr. R. Lousberg.

Matthias Walter. **Multi-methodological approaches to investigate lower urinary tract function in health and disease.** Supervisors: Prof.dr. Ph.E.V.A. van Kerrebroek; Prof.dr. G.A. van Koeveinge; Prof.dr. A. Curt, Zürich, CH.

Lalit Gupta. **Inhomogeneities in spontaneous brain fluctuations.** Supervisors: Prof.dr. W.H. Backes; Prof.dr. P.A.M. Hofman. Co-supervisor: Dr. J.F.A. Jansen.

Chaitra Jayadev. **Impact of imaging the pediatric retina.** Supervisor: Prof.dr. C.A.B. Webers. Co-supervisor: Dr. N.J.C. Bauer; Dr. A. Vinekar.

Annelie Klippel. **Navigating through complexity; processes and mechanisms underlying the development of psychosis.** Supervisors: Prof.dr. I. Myin-Germeys, KU-Leuven; Prof.dr. M.C. Wichers, UMC Groningen. Co-supervisor: Dr. U. Reininghaus.

Kürşat Altınbaş, **Reconstructing the Diagnostic Framework of Bipolarity**. Supervisor: Prof.dr. J. van Os. Co-supervisor: Dr. I.S. Gülöksüz.

Andrea J.R. Balthasar, **Eyes of the needle; Spectral tissue sensing, an innovative technology for detecting various tissue types during percutaneous needle-based procedures in locoregional anesthesia and pain medicine**. Supervisor: Prof.dr. M. van Kleef. Co-supervisor: Dr. G.-J. van Geffen, Radboud UMC Nijmegen.

Walmari Pilz, **Shedding light on oropharyngeal dysphagia in myotonic dystrophy type 1**. Supervisor: Prof.dr. B. Kremer. Co-supervisors: Dr. L.W.J. Baijens; Dr. V. Lima Passos.