COGNITIVE FUNCTIONING AND MICROVASCULAR DISEASE

Sophie Heringa

Cover image: Kuin Heuff
Lay-out: Teuntje van de Wouw
Printed by: Ipskamp Drukkers B.V.
ISBN: 978-94-6259-020-5

© 2014 S.M. Heringa
All rights reserved. No part of this publication may be reproduced or transmitted in any form or by any means, without permission in writing from the author. The copyright of the articles that have been published has been transferred to the respective journals.

COGNITIVE FUNCTIONING AND MICROVASCULAR DISEASE

Cognitief functioneren en microvasculaire schade

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 4 februari 2014 des middags te 4.15 uur

door

Sophia Maria Heringa
geboren op 21 mei 1984 te Enschede

Promotoren: Prof. dr. G.J. Biessels
Prof. dr. L.J. Kappelle

Co-promotor: Dr. E. van den Berg

The studies described in this thesis were supported by the Internationale Stichting Alzheimer Onderzoek, grant 10506.

Financial support by the Dutch Heart Foundation and Stichting Het Heringafonds for the publication of this thesis is gratefully acknowledged.

Additional financial support was generously provided by Alzheimer Nederland (Bunnik), the Internationale Stichting Alzheimer Onderzoek, and Boehringer Ingelheim.

voor Bram en Reina

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Vascular damage and cognitive impairment and dementia

Cognitive impairment and dementia form a major health issue, affecting a considerable proportion of the aging population. Cerebral vascular damage is increasingly recognized as one of the main causes of cognitive decline in aging and dementia.[1] Vascular risk factors, including hypertension, diabetes mellitus, obesity, dyslipidemia, and smoking, not only predispose to cardiovascular disease and stroke,[2] but are also related to the entire spectrum of cognitive dysfunctioning, from subtle decrements[3] to dementia.[4]

Vascular damage and Alzheimer type pathology

Neurodegenerative processes are another main cause of cognitive deterioration in older people. Prototypes of the resulting neuropathologies are the two core pathological hallmarks of Alzheimer's disease (AD) – amyloid plaques and neurofibrillary tangles, respectively.[5] The presence of these pathological hallmarks is related to the severity of cognitive impairment or dementia at the time of death,[6] although this association is also largely dependent on other factors such as age.[7] How these AD type pathological processes and their underlying disease processes lead to grave cognitive disturbances is still not completely understood.

Evidence is growing for a crucial role of vascular damage in AD. For instance, vascular risk factors contribute to development of AD.[8] Furthermore, autopsy studies have revealed that a majority of older people exhibit a mixed profile of vascular damage and AD pathology.[9] Both types of pathology contribute to normal age-related cognitive decline and to accelerated cognitive decline resulting in dementia.[10,11] These findings have led to a revision of the former categorical view of vascular dementia and AD as two separate entities. A dimensional view on cognitive decline and dementia seems more accurate, with a spectrum ranging from patients with pure vascular dementia to patients with pure AD and including a large majority of patients with contributions from both pathologies (Figure 1, reproduced from Viswanathan et al.[8]). This view poses new questions for the research field. A current challenge is to explore the role of vascular disease in causing cognitive impairment, by itself or in interaction with AD. This is of particular interest because risk factors for vascular disease are potentially modifiable, which may give a lead for prevention and treatment of late-life cognitive decline. Furthermore, a proper delineation of vascular

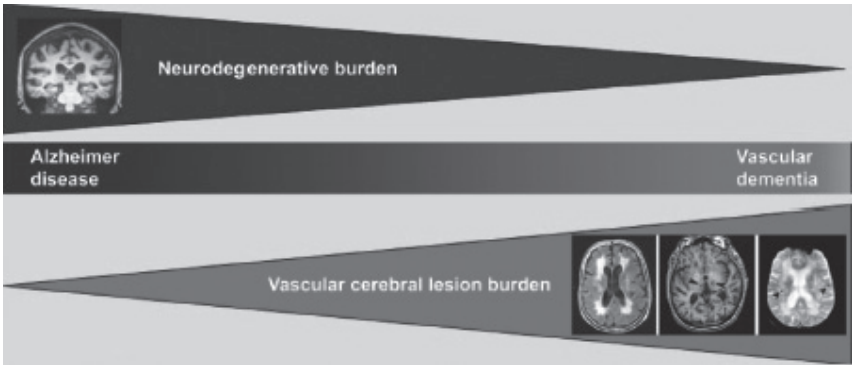


Figure 1. Schematic diagram of AD and vascular dementia that fall on a continuous spectrum of disease, composed of features of both neurodegenerative and vascular pathologies (reproduced from Viswanathan et al.[8])

damage in an individual may help to detect persons at risk for cognitive problems or may aid in predicting the course of cognitive deterioration. The concept of vascular cognitive impairment (VCI) was introduced to refer to all forms of mild to severe cognitive impairment associated with, and presumed to be caused by, cerebrovascular disease.[12]

Microvascular disease

The relation between vascular risk factors, vascular disease, and cognitive functioning is complex and has several underlying mechanisms. Evidently, large vessel ischemic strokes and intracerebral hemorrhages may exert a direct effect on cognitive functioning.[13] However, not only large blood vessel disease may have an impact on the brain. The cerebral microvasculature is also fundamentally important for brain functioning, and damage to these small vessels is often associated with cognitive disturbances.[14] Conventional techniques to assess the vascular damage to the brain include magnetic resonance imaging (MRI). With an MRI scan, it is possible to visualize the manifestations of cerebral small vessel disease, such as white matter hyperintensities, lacunar infarcts, or microbleeds. Each of these manifestations is related to cognitive impairments.[15–17] This relation with cognitive functioning is present in normal aging, but also in conditions such as vascular dementia and AD. Thus, microvascular damage is important for the entire spectrum

of cognitive functioning, from subtle decrements in normal aging to mild cognitive impairment (MCI) and frank dementia, and it is important to better understand this relation. At the level of the older population, markers of microvascular disease have not yet been studied extensively in relation to detailed cognitive assessment covering multiple domains. Furthermore, measures capturing the vascular burden in AD have not been fully established. Microvascular brain lesions are promising novel markers of this burden, that can be used in etiological and treatment studies. In the research described in this thesis, we applied different methods of assessing microvascular disease and we examined the relation with cognitive functioning in normal aging and in patients with amnestic MCI (aMCI; considered to represent a transitional stage between normal aging and AD[18]) or early AD.

Systemic markers of microvascular disease

Microvascular disease is a systemic process, which can affect all parts of the body. Presence of microvascular damage outside the brain may thus be related to vascular damage inside the brain and, consequently, to cognitive functioning. In this context, the eye and the kidney may offer windows on the vasculature of the brain. The vessels of the retina in particular are anatomically and physiologically related to those of the brain and share a similar vulnerability to vascular risk factor exposure.[19,20] Along these lines, microvascular changes in the kidney may also reflect exposure to vascular risk and an individual's vulnerability to microvascular damage. Because of these close links, direct or indirect measurements of vascular damage in these organs may convey information about vascular damage in the brain, and hence about cognitive impairment.

Vascular abnormalities in the retina are detected using fundus photography or scopy. Retinal lesions such as microhemorrhages, microaneurysms, arteriovenous nicking, or arteriolar narrowing (Figure 2) are manifestations of microvascular disease that can be found in the general population. The prevalence of such abnormalities in the general aging population varies from 2 to 15%.[21] Microvascular damage in the kidney is assessed by measuring the ratio between urine albumin and creatinine. An albumin-to-creatinine ratio of ≥2.0 mg/ml is classified as albuminuria[22,23] and implies suboptimal functioning

of the small vessels in the kidney. The prevalence of albuminuria is similar to that of retinal microvascular changes and varies from 10 to 16%.[24] Another way of measuring systemic microvascular disease is by determining endothelial dysfunction or low-grade inflammation. Presence of low-grade inflammation or endothelial dysfunction is related to cardiovascular risk factors and disturbed microcirculation.[25] It is reflected in modest elevated serum levels of several circulating biomarkers. These markers include for low-grade inflammation acute-phase reactants C-reactive protein and serum amyloid A, cytokines tumor necrosis factor alpha, interkleukin-6, and interleukin-8, the enzyme myeloperoxidase, and the adhesion molecule soluble intercellular adhesion molecule-1.[26,27] For endothelial dysfunction they include von Willebrand factor, soluble vascular cell adhesion molecule-1, soluble endothelial selectin, soluble thrombomodulin, and soluble intercellular adhesion molecule-1.[26,27] These systemic urine, retinal, or serum biomarkers of microvascular damage can be measured in a relatively cost-effective and undemanding way.

Thus, retinal microvascular changes, albuminuria, and endothelial dysfunction/low-grade inflammation are various manifestations of end organ damage or systemic microvascular disease that may mark the presence of vascular disease in the brain and that can easily be measured. We hypothesize that presence of these markers is associated with worse cognitive functioning.[28] The first part of this thesis includes an overview of the literature on this association for retinal microvascular markers. Furthermore, studies are described that test our hypothesis in an older population-based sample. All participants underwent measurements of biomarkers of microvascular damage, extensive neuropsychological examination, and a detailed vascular risk factor profile assessment. We investigated whether markers of microvascular damage outside the brain were

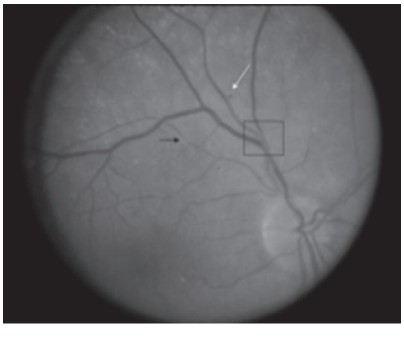


Figure 2. Fundus photograph of the retina, with focal arteriolar narrowing (black arrow), microaneurysm (white arrow), arteriovenous nicking (box)

cross-sectionally or prospectively related to worse cognitive functioning. Such an association would provide support for (micro)vascular damage as a possible cause of cognitive impairment within an individual. Besides the etiological aspect of this association, these biomarkers may have a diagnostic or prognostic value. Both from a clinical and research perspective, it is important to identify people at risk of having or developing cognitive impairment or dementia. These are the persons who might need monitoring of their cognitive abilities, or who might benefit from more intensive treatment of vascular risk factors. For the purpose of detecting this subgroup, screening instruments may be useful. As such, systemic biomarkers of microvascular disease in urine, retina, or serum are potential candidates for such a screening instrument, as they have a clear advantage over other potential diagnostic or prognostic markers for cognitive impairment that are measured with MRI, PET biomarkers, or biomarkers in the cerebral spinal fluid.[29–31]

Cerebral markers of microvascular disease in dementia

Cerebrovascular lesions such as white matter hyperintensities, lacunar infarcts, and (large) cortical infarcts are observed in a substantial proportion of patients with dementia, also in those with AD. However, these lesions, which are readily detectable with conventional MRI, do not capture the whole burden of vascular damage.

In this light, cerebral microbleeds are attracting increasing attention. Microbleeds are tiny collections of blood-breakdown products that have leaked by nearby damaged small vessels.[32] They are radiologically defined as small, rounded, homogeneous, hypointense lesions on T2*- weighed gradient-recalled echo (T2*-GRE) and related MRI sequences sensitive to magnetic susceptibility (Figure 3).[33] They are related to impaired cognition in healthy older people (e.g.[17]), and in patients with vascular burden (e.g.[34]). In patients with AD, however, this relation is less evident. Most studies thus far have not found an association between presence of microbleeds and concurrent Mini-Mental State Examination (MMSE) scores (e.g.[35]), although multiple microbleeds were associated with worse cognitive functioning in one study.[36]

Cortical microinfarcts are another expression of microvascular damage in patients with AD. They are commonly described in neuropathology studies,

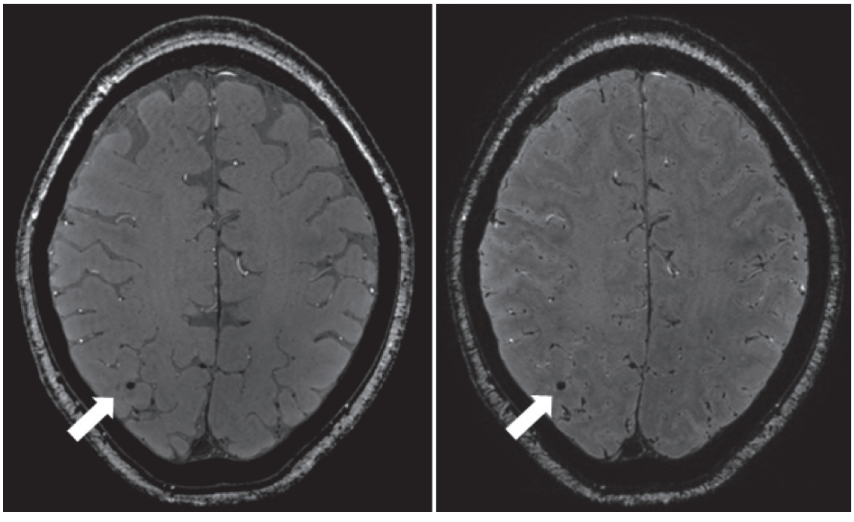


Figure 3. Microbleed at 7 Tesla MRI (dual echo GRE)

particularly in patients with vascular dementia, AD, or cerebrovascular disease.[37] Cortical microinfarcts are defined as sharply delimited microscopic regions of cellular death or tissue necrosis, sometimes with cavitation (i.e., a central fluid-filled cavity).[38] They occur throughout the brain, and in the majority of cases multiple microinfarcts are observed, with estimates of up to a thousand within a single individual.[39] In autopsy studies, cortical microinfarcts are related to an increased risk of cognitive decline and dementia, in people with high and low levels of AD type pathology, and in people with or without comorbid macroinfarcts.[38]

Thus, microbleeds and microinfarcts are crucial to investigate the role of vascular pathology in the progression of AD. Part two of this thesis includes studies on cerebral microbleeds and cerebral cortical microinfarcts in patients with aMCI or early AD, in relation to the clinical profile.

The association between microbleeds and cognitive dysfunctioning is complex, and has several possible explanations. Microbleeds may have a direct effect of on the brain, or should rather be regarded as a marker of other vascular damage in the brain that causes cognitive impairment. For example, it is conceivable that microbleeds or microbleed-related pathologies affect cognitive functioning by disrupting white matter brain connectivity. To gain further insight

in these relations, we use diffusion tensor imaging (DTI) to reconstruct the brain network,[40,41] and graph theoretical analysis to quantify the quality or efficiency of the network.[42] Using these techniques, we investigate whether microbleeds are associated with disruptions of the white matter microstructure and organization, and to cognitive functioning, in patients with aMCI or early AD.

In patients with AD, assessment of microbleeds with conventional MRI yields prevalence estimates that are lower than would be expected based on autopsy studies. In vivo prevalence estimates of microbleeds are around 16-32%,[43] whereas cerebral amyloid angiopathy, the presumed main neuropathological correlate of microbleeds in AD, is observed in 82-98% of patients.[44] The actual prevalence may thus be much higher. This is a question that is addressed in this thesis, using ultra high field strength MRI. The University Medical Center Utrecht owns a 7 Tesla MRI scanner, as one of three medical centers in the Netherlands. Due to the high field strength of this system, scans can be acquired at a high resolution, and paramagnetic substances such as hemosiderin deposits induce large susceptibility effects.[45] These advantages over conventional lower field strengths of 1.0 – 3.0 Tesla improve the detection of microbleeds. Therefore, we apply this novel technique to measure more accurately the prevalence of microbleeds in patients with aMCI or early AD.

Until recently, imaging microinfarcts in vivo was not possible. Last year, our group accomplished this for the first time, using 7 Tesla MRI.[46] In this thesis, we explored the possibilities of investigating these lesions in patients with aMCI or early AD in vivo and relating them to a clinical profile, including vascular risk factors and cognition.

In sum, by linking the presence and number of cerebral microvascular lesions to other vascular and clinical parameters, we aim to take an important step forward in studying the microvascular burden in AD, which can be applied in etiological and treatment studies.

Outline of the thesis

In **part I**, the relation is investigated between biomarkers of systemic microvascular disease and cognitive functioning. **Chapter 2** provides a systematic review of the literature on retinal vascular changes in relation to

dementia, cognitive functioning, and brain imaging abnormalities. Chapters 3, 4, and 5 are based on data from a sample of older, non-demented individuals, participating in the population-based Hoorn Study. **Chapter 3** describes the relation between albuminuria and cognitive functioning, both prospectively and cross-sectionally. **Chapter 4** describes the relation between low-grade inflammation and endothelial dysfunction and cognitive functioning, both prospectively and cross-sectionally. **Chapter 5** describes the cross-sectional relation between retinopathy and cognitive functioning.

In **part II**, the relation is investigated between markers of cerebral microvascular damage and cognitive functioning and clinical outcome, within patients with aMCI or early AD. We explore new imaging techniques to study microvascular lesions. In **chapter 6**, we use 3 Tesla MRI and DTI to examine the relation between microbleeds, the microstructure and organization of the cerebral network, and cognitive functioning in patients with aMCI or early AD. In **chapter 7**, we use 7 Tesla MRI to assess the presence and number of microbleeds in patients with aMCI or early AD, relative to older controls. In **chapter 8**, we use 7 Tesla MRI to assess the presence and number of cortical microinfarcts in patients with aMCI or early AD, relative to older controls.

We were also interested in taking an in-depth look at a cognitive function that is often affected in patients with aMCI or early AD. In this group, a deficit in spatial navigation is commonly observed, which poses a burden on patients and caregivers.[47] In **part III**, we investigate the ability of spatial navigation in patients with aMCI or early AD, compared to controls. In **chapter 9**, we perform an experimental task that measures three aspects of spatial navigation and we examine the relation with functioning on other cognitive domains.

Finally, in **chapter 10**, the results presented in this thesis are discussed and future directions for research and clinical practice are proposed.

REFERENCES

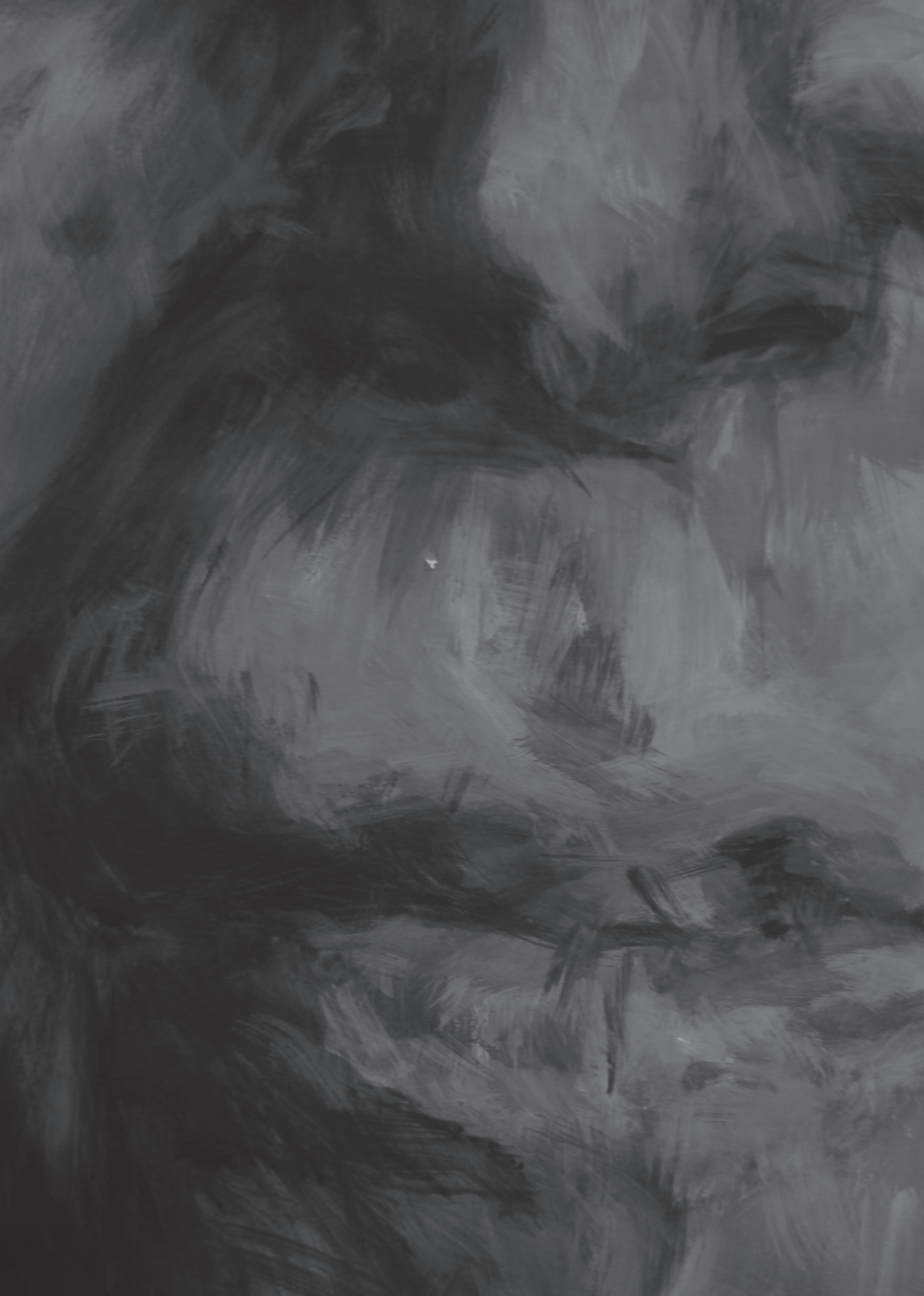
1. Hachinski V. Shifts in thinking about dementia. *Journal of the American Medical Association*. 2008;300:2172–3.
2. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation*. 2012;125:e2–e220.
3. Van den Berg E, Kloppenborg RP, Kessels RPC, Kappelle LJ, Biessels GJ. Type 2 diabetes mellitus, hypertension, dyslipidemia and obesity: A systematic comparison of their impact on cognition. *Biochimica et biophysica acta*. 2009;1792:470–81.
4. Kloppenborg RP, van den Berg E, Kappelle LJ, Biessels GJ. Diabetes and other vascular risk factors for dementia: which factor matters most? A systematic review. *European journal of pharmacology*. 2008;585:97–108.
5. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet*. 2011;377:1019–31.
6. Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, Castellani RJ, Crain BJ, Davies P, Del Tredici K, Duyckaerts C, Frosch MP, Haroutunian V, Hof PR, Hulette CM, Hyman BT, Iwatsubo T, Jellinger KA, Jicha GA, Kövari E, Kukull WA, Leverenz JB, Love S, Mackenzie IR, Mann DM, Masliah E, McKee AC, Montine TJ, Morris JC, Schneider JA, Sonnen JA, Thal DR, Trojanowski JQ, Troncoso JC, Wisniewski T, Woltjer RL, Beach TG. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. *Journal of neuropathology and experimental neurology*. 2012;71:362–81.
7. Savva GM, Wharton SB, Ince PG, Forster G, Matthews FE, Brayne C. Age, neuropathology, and dementia. *The New England journal of medicine*. 2009;360:2302–9.
8. Viswanathan A, Rocca WA, Tzourio C. Vascular risk factors and dementia: how to move forward? *Neurology*. 2009;72:368–74.
9. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology*. 2007;69:2197–204.

10. Wilson RS, Leurgans SE, Boyle PA, Schneider JA, Bennett DA. Neurodegenerative basis of age-related cognitive decline. *Neurology*. 2010;75:1070–8.
11. Bennett DA, Wilson RS, Boyle PA, Buchman AS, Schneider JA. Relation of neuropathology to cognition in persons without cognitive impairment. *Annals of neurology*. 2012;72:599–609.
12. O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, Bowler J V, Ballard C, DeCarli C, Gorelick PB, Rockwood K, Burns A, Gauthier S, DeKosky ST. Vascular cognitive impairment. *Lancet neurology*. 2003;2:89–98.
13. Nys GMS, van Zandvoort MJE, de Kort PLM, Jansen BPW, de Haan EHF, Kappelle LJ. Cognitive disorders in acute stroke: prevalence and clinical determinants. *Cerebrovascular diseases*. 2007;23:408–16.
14. Román GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC. Subcortical ischaemic vascular dementia. *Lancet neurology*. 2002;1:426–36.
15. Pantoni L, Poggesi A, Inzitari D. The relation between white-matter lesions and cognition. *Current opinion in neurology*. 2007;20:390–7.
16. Edwards JD, Jacova C, Sepehry AA, Pratt B, Benavente OR. A quantitative systematic review of domain-specific cognitive impairment in lacunar stroke. *Neurology*. 2013;80:315–22.
17. Poels MMF, Ikram MA, van der Lugt A, Hofman A, Niessen WJ, Krestin GP, Breteler MMB, Vernooij MW. Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. *Neurology*. 2012;78:326–33.
18. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Archives of neurology*. 1999;56:303–8.
19. Wong TY, Klein R, Klein BE, Tielsch JM, Hubbard L, Nieto FJ. Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. *Survey of ophthalmology*. 2001;46:59–80.
20. Wong TY, McIntosh R. Systemic associations of retinal microvascular signs: a review of recent population-based studies. *Ophthalmic & physiological optics*. 2005;25:195–204.
21. Grosso A, Cheung N, Veglio F, Wong TY. Similarities and differences in early retinal phenotypes in hypertension and diabetes. *Journal of hypertension*. 2011;29:1667–75.

22. Jager A, Kostense PJ, Nijpels G, Heine RJ, Bouter LM, Stehouwer CDA. Microalbuminuria is strongly associated with NIDDM and hypertension, but not with the insulin resistance syndrome: the Hoorn Study. *Diabetologia*. 1998;41:694–700.
23. Gatling W, Knight C, Mullee MA, Hill RD. Microalbuminuria in diabetes: a population study of the prevalence and an assessment of three screening tests. *Diabetic medicine*. 1988;5:343–7.
24. Nitsch D, Grams M, Sang Y, Black C, Cirillo M, Djurdjev O, Iseki K, Jassal SK, Kimm H, Kronenberg F, Oien CM, Levey AS, Levin A, Woodward M, Hemmelgarn BR. Associations of estimated glomerular filtration rate and albuminuria with mortality and renal failure by sex: a meta-analysis. *BMJ*. 2013;346:f324.
25. Granger DN, Rodrigues SF, Yildirim A, Senchenkova EY. Microvascular responses to cardiovascular risk factors. Microcirculation. 2010;17:192–205.
26. Borissoff JI, Spronk HMH, ten Cate H. The hemostatic system as a modulator of atherosclerosis. *The New England journal of medicine*. 2011;364:1746–60.
27. Ross R. Atherosclerosis--an inflammatory disease. *The New England journal of medicine*. 1999;340:115–26.
28. Thompson CS, Hakim AM. Living beyond our physiological means: small vessel disease of the brain is an expression of a systemic failure in arteriolar function: a unifying hypothesis. *Stroke*. 2009;40:e322–30.
29. Moghekar A, Li S, Lu Y, Li M, Wang M-C, Albert M, O'Brien R. CSF biomarker changes precede symptom onset of mild cognitive impairment. *Neurology*. 2013;
30. Shaffer JL, Petrella JR, Sheldon FC, Choudhury KR, Calhoun VD, Coleman RE, Doraiswamy PM. Predicting cognitive decline in subjects at risk for Alzheimer disease by using combined cerebrospinal fluid, MR imaging, and PET biomarkers. *Radiology*. 2013;266:583–91.
31. Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet neurology*. 2010;9:119–28.
32. Fazekas F, Kleinert R, Roob G, Kleinert G, Kapeller P, Schmidt R, Hartung HP. 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. *American journal of neuroradiology*. 1999;20:637–42.

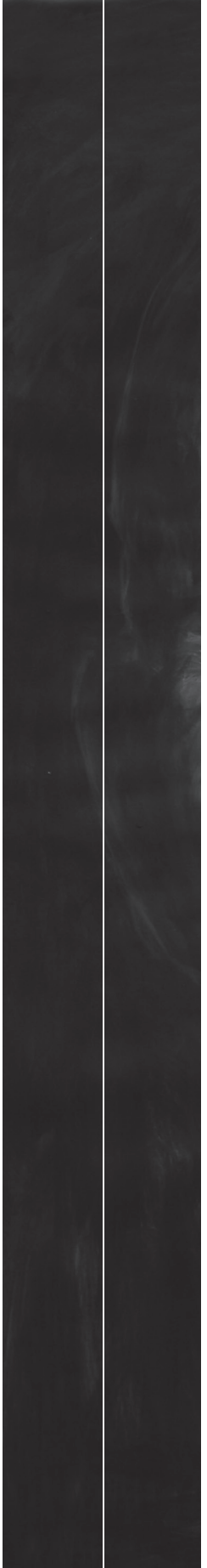
33. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, Launer LJ, Van Buchem MA, Breteler MM. Cerebral microbleeds: a guide to detection and interpretation. *Lancet neurology*. 2009;8:165–74.
34. Van Norden AGW, van den Berg HAC, de Laat KF, Gons RAR, van Dijk EJ, de Leeuw F-E. Frontal and temporal microbleeds are related to cognitive function: the Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Cohort (RUN DMC) Study. *Stroke*. 2011;42:3382–6.
35. Pettersen JA, Sathiyamoorthy G, Gao F-Q, Szilagyi G, Nadkarni NK, St George-Hyslop P, Rogaeva E, Black SE. Microbleed topography, leukoaraiosis, and cognition in probable Alzheimer disease from the Sunnybrook dementia study. *Archives of neurology*. 2008;65:790–5.
36. Goos JDC, Kester MI, Barkhof F, Klein M, Blankenstein MA, Scheltens P, van der Flier WM. Patients with Alzheimer disease with multiple microbleeds: relation with cerebrospinal fluid biomarkers and cognition. *Stroke*. 2009;40:3455–60.
37. Brundel M, de Bresser J, van Dillen JJ, Kappelle LJ, Biessels GJ. Cerebral microinfarcts: a systematic review of neuropathological studies. *Journal of cerebral blood flow and metabolism*. 2012;32:425–36.
38. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesions. *Lancet neurology*. 2012;11:272–82.
39. Westover MB, Bianchi MT, Yang C, Schneider JA, Greenberg SM. Estimating cerebral microinfarct burden from autopsy samples. *Neurology*. 2013;80:1365–9.
40. Tournier J-D, Mori S, Leemans A. Diffusion tensor imaging and beyond. *Magnetic Resonance in Medicine*. 2011;65:1532–56.
41. Hagmann P, Cammoun L, Gigandet X, Meuli R, Honey CJ, Wedeen VJ, Sporns O. Mapping the structural core of human cerebral cortex. *PLoS biology*. 2008;6:e159.
42. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. Nature reviews. *Neuroscience*. 2009;10:186–98.
43. Cordonnier C, van der Flier WM. Brain microbleeds and Alzheimer's disease: innocent observation or key player? *Brain*. 2011;134:335–44.
44. Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. *Journal of neural transmission*. 2002;109:813–36.
45. Conijn MMA, Geerlings MI, Luijten PR, Zwanenburg JJM, Visser F, Biessels GJ, Hendrikse J. Visualization of cerebral microbleeds with dual-echo T2*-weighted magnetic resonance imaging at 7.0 T. *Journal of magnetic resonance imaging*. 2010;32:52–9.

46. Van Veluw SJ, Zwanenburg JJM, Engelen-Lee J, Spliet WGM, Hendrikse J, Luijten PR, Biessels GJ. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. *Journal of cerebral blood flow and metabolism*. 2013;33:322–9.
47. Pai M, Jacobs WJ. Topographical disorientation in community-residing patients with Alzheimer's disease. *International journal of geriatric psychiatry*. 2004;19:250–5.



PART I

SYSTEMIC MARKERS OF MICROVASCULAR DISEASE AND COGNITIVE FUNCTIONING



CHAPTER 2

ASSOCIATIONS BETWEEN RETINAL MICROVASCULAR CHANGES AND DEMENTIA, COGNITIVE FUNCTIONING, AND BRAIN IMAGING ABNORMALITIES: A SYSTEMATIC REVIEW

SM Heringa, WH Bouvy, E van den Berg,
AC Moll, LJ Kappelle, GJ Biessels

Published in: Journal of Cerebral Blood Flow & Metabolism
2013;33:983–995.

ABSTRACT

BACKGROUND Retinal microvascular changes can be visualized non-invasively and have been associated with cognitive decline and brain changes in relation to aging and vascular disease.

METHODS We systematically reviewed studies, published between 1990 and November 2012, on the association between retinal microvascular changes and dementia, cognitive functioning and brain imaging abnormalities, in the context of aging and vascular risk factors.

RESULTS In cross-sectional studies (k=26), retinal microvascular changes were associated with presence of dementia (range of odds ratios 1.17;5.57), with modest decrements in cognitive functioning in non-demented people (effect sizes -0.25;0.03), and with brain imaging abnormalities, including atrophy and vascular lesions (odds ratios 0.94;2.95). Longitudinal studies were more sparse (k=9) and showed no consistent associations between retinal microvascular changes and dementia or cognitive dysfunctioning 3-15 years later (odds ratios and hazard ratios 0.77;1.55). However, there were indications of prospective associations with brain imaging abnormalities (odds ratios 0.81;3.19).

CONCLUSION Particularly in cross-sectional studies there is a correlation between retinal microvascular changes and dementia, cognitive impairment, and brain imaging abnormalities. Associations are strongest for more severe retinal microvascular abnormalities. Retinal microvascular abnormalities may offer an important window on the brain for etiological studies.

INTRODUCTION

Vascular disease is an important cause of dementia and cognitive decline in older people.[1] Vascular damage in the brain often co-occurs with vascular disease elsewhere in the body, probably reflecting common underlying pathophysiological processes.[2] This concerns damage to both large and small vessels. Hence, markers of micro- or macrovascular disease outside the brain may convey information about vascular abnormalities in the brain and, consequently, about late-life cognitive functioning. In this context, the vessels of the retina are of particular interest for two reasons: they can be directly visualized non-invasively and they share anatomic, embryologic, and physiologic characteristics with the cerebral microvasculature.[3,4]

Microvascular changes in the retina are associated with hypertension, inflammation, diabetes, stroke and cardiovascular disease, as previously described in review articles.[5,6] Also, associations between retinal microvascular changes and dementia risk, cognitive functioning, or brain imaging abnormalities have been reviewed.[7,8] However, to our knowledge, no integrated, comprehensive, quantitative overview of these associations has been published yet.

We hypothesized that retinal microvascular changes are a marker of cerebral abnormalities, in particular vascular lesions and atrophy, and as such may be linked to dementia or cognitive impairment. Therefore, the aim of this chapter is to provide a systematic review of the literature on the association between retinal microvascular changes and dementia or cognitive functioning and brain imaging abnormalities, in the context of aging and vascular risk factors.

METHODS

Identification of studies

This systematic review was performed according to the methodology of the PRISMA statement.[9,10] We aimed to include all published studies that provided analyses of the relation between retinal microvascular changes and the following outcome measures: dementia or cognitive functioning and brain imaging abnormalities. We applied the following inclusion criteria: the study (1) reported on measures of retinal microvascular changes in relation to dementia

or cognitive functioning or brain imaging abnormalities, (2) was published after 1990, (3) had a population-based design or consisted of participants selected on the presence of specific vascular risk factors or vascular disease, (4) reported on a sample size of ≥ 250 for population-based cohorts and ≥ 50 for patient-based cohorts, (5) reported on participants with a mean age ≥ 50 and adjusted for age in their analyses. A minimum sample size was chosen because in small studies the statistical power may be insufficient to reliably evaluate the relation between retinal microvascular changes and the outcome measures. A minimum age was required because this review focuses on the relation between retinal microvascular changes and the outcomes in the context of aging. Any method that was used to assess retinal vasculature, cognition or brain imaging abnormalities was accepted. We excluded studies that selected participants on the presence of retinal microvascular changes or the outcomes (i.e. dementia and cognitive functioning or brain imaging abnormalities). We also excluded studies that investigated type 1 diabetes, because in these patients the role of vascular risk factors and aging is different from the general population or patients with type 2 diabetes.

Medline (1990 to November 22, 2012) and cross references from included papers were used to identify relevant papers. The search was limited to papers that were written in English. Searches were performed separately for cognitive and imaging outcome measures. We used the following search terms: ('retina' and ('microvascular', 'arteriolar', 'venular', or 'vascular') or 'retinopathy') in combination with ('cognition', 'dementia', or 'memory') and, separately, in combination with (('brain' or 'cerebral') and ('mri' or 'ct' or 'imaging' or 'lesion')) and ('volume', 'atrophy', 'white matter', 'hemorrhage', 'bleeding', 'microbleeds', 'infarct', 'stroke', or 'subcortical'), all terms in full or truncated versions. Titles and abstracts were scanned and potentially eligible papers were collected in full-text versions. SMH and WHB independently judged all eligible papers according to the inclusion criteria.

Included studies

For dementia and cognitive functioning the search yielded 278 hits, of these 16 were included.[11–26] One additional study[27] was added after search of reference lists. For brain imaging abnormalities the search yielded 228 hits, of these 15 were included.[4,23,26,28–40] Multiple papers from the same study population were

included only if different types of retinal microvascular changes or outcome measures were examined in separate papers. We excluded two population-based studies because they reported on a sample size of 207[41] or 96[42]. All patient-based studies had a sample size over 50.

Data extraction

We extracted study characteristics, the ways in which retinal microvascular changes and the outcomes were measured, and factors that were adjusted for in analyses (Table 1). We compiled retinal, cognitive and brain imaging variables into categories that optimally accommodated the data extracted from the included studies.

Data on the cognitive and brain imaging outcomes are presented in Tables 2 a through c. When applicable, results are presented that were adjusted for demographics and vascular risk factors. For dichotomous outcome variables results are presented as odds ratios (ORs) or hazard ratios (HRs). With regard to dementia, data are presented for any dementia, rather than for a subtype such as Alzheimer's disease or vascular dementia. For continuous variables standardized regression coefficients (betas) were extracted, or means and standard deviations were converted into Cohen's *d*[43] to provide estimates of effect sizes. Median ORs or effect sizes are presented in case multiple results were reported for the same measure. In tables 2 a through c, negative effect sizes or betas indicate worse cognition or more brain imaging abnormalities in the group with (more) retinal microvascular changes. Effect sizes <0.2 are considered small, 0.2-0.8 medium and > 0.8 large.[43]

The results of studies that did not present data that could be converted into effect sizes or ORs / HRs are presented by means of direction of effect. Associations of elevated levels of retinal microvascular changes with worse cognition or more brain imaging abnormalities were indicated by '–', association with better cognition or less brain imaging abnormalities were indicated by '+', and no statistically significant association between retinal microvascular changes and cognition or brain imaging abnormalities was indicated by '='. Results from cross-sectional and longitudinal studies are presented separately, as well as population-based studies and studies with patients with vascular risk factors.

We intended to perform meta-analyses, using random effect models,

to pool the findings of studies that were sufficiently homogeneous with regard to study design, retinal measures and cerebral outcome measures. Pooled data are presented only when effect sizes between studies were not heterogeneous (examined with I^2 statistic, values near 75% or greater indicate high heterogeneity[44]). Such meta-analyses only proved to be possible for the cognitive outcome measure of any retinopathy.

RESULTS

Measurements

Retinal measurements

Photographs of the retina were taken after pharmacological pupil dilation, except in the ARIC and CHS studies, in which dark adaptation was used.[12,13,15,29–33,40] In the J-EDIT study[27] no photographs were taken, but funduscopy was performed after dilation. Studies reported on the following retinal measures: any retinopathy, arteriovenous nicking, focal narrowing, arteriolar narrowing, and venular widening. Retinopathy, focal narrowing, and arteriovenous nicking were graded visually from photographs. Authors defined any retinopathy as present if any of the following lesions were observed: blot or flameshaped hemorrhages, microaneurysms, soft exudates (cotton wool spots), hard exudates, optic disc swelling, intraretinal microvascular abnormalities, venous beading, new vessels at the disk or elsewhere, vitreous hemorrhage, or laser photocoagulation scars. Three population-based studies (BMES,[14] SiMES,[22] and WHI[23]) used a grading scale for diabetic retinopathy that was derived from the Early Treatment Diabetic Retinopathy Study (ETDRS).[45] The definition of any retinopathy in studies comprising patients with diabetes was extended to include fibrous proliferations, preretinal hemorrhage, and retinal detachment.[24,27]

Retinal arteriolar and venular caliber measurements were performed via computer-assisted techniques and summarized into central retinal artery equivalents (CRAE) or central retinal venular equivalents (CRVE).[46] Some studies used caliber measurements as continuous measures,[17–19,34] whereas others categorized these into quartiles,[13,14,17] quintiles,[4,12,15,29,30,33,37] or deciles.[40] Narrowest arteriolar and widest venular calibers were set against their reference groups. Any retinopathy was assessed in most studies (eight out of nine

population-based cohorts and all six patient-based cohorts). Arteriovenous nicking or focal narrowing were assessed in three population-based cohorts and one patient-based cohort. Measures of vessel caliber were assessed in seven population-based and two patient-based cohorts.

Cognitive outcome measures

The relation between retinal microvascular changes and dementia or cognitive functioning was assessed in nine population-based cohorts: AGES,[11] ARIC,[12,13] BMES,[14] CHS,[15,40] LALES,[17] Lothian Birth Cohort 1921,[18] Rotterdam Study,[19,20] SiMES,[21,22] WHI,[23] and in three cohorts of patients with diabetes: ET2DM,[24,25] J-EDIT,[27] UDES.[26] Dementia as an outcome measure was used in three population-based cohorts. Diagnostic procedures for dementia assessment consisted of cognitive screening of all participants and a subsequent elaborate examination for the presence of dementia in those who screened positive. A multidisciplinary team of physicians (neurologist, psychiatrist, geriatrician and/or neuroradiologist) and psychologists determined final dementia diagnosis, based on standardized criteria for dementia,[47] Alzheimer's disease,[48] or vascular dementia.[49] Four population-based cohorts and one cohort of patients with diabetes reported on cognitive impairment as an outcome measure. Impairment was considered present if performance was below a predefined cut off or deviated from normal within the studied population (≤2 SD below the mean, or <1st decile). Five population-based studies and two type 2 diabetes studies reported on one to four of the following cognitive domains: memory, information processing speed, executive functioning or abstract reasoning[50] (see appendix for cognitive tests per domain). Three population-based studies and one diabetes study reported on change in cognitive functioning over time. In one study cognitive domains were measured at four time points, outcome measures were rates of decline per domain and risk of belonging to the 10% most rapid decliners.[13] In another study, risk of incident dementia was rated[19,20] and in yet another Modified MMSE (3MSE) scores of each annual time point were compared between groups.[23] One study in patients with diabetes expressed cognitive decline as a regression based index, controlling for baseline demographics, baseline cognition and decline in control participants.[26]

Table 1. Description of included studies

Study cohort	Report	N (% of eligible)	Age[a]	Male	Design
Population-based					
Age, Gene/ Environment Susceptibility (AGES)-Reykjavik Study	[39]	4218 (73%)	76	43%	Cross-sectional
	[28]	4176 (72%)	76	42%	Cross-sectional
	[11]	3906 (68%)	76	42%	Cross-sectional
Atherosclerosis Risk in Communities Study (ARIC)	[12]	8734 (86%)	54	48%	Cross-sectional
	[29]	1684 (58%)	62	40%	Cross-sectional
	[30]	1684 (58%)	62	40%	Cross-sectional
	[4]	1684 (58%)	62	40%	Cross-sectional
	[13]	803 (93%)	58	40%	14 years follow-up
	[31]	810 (28%)	62	40%	10 years follow-up
	[32]	810 (28%)	62	40%	10 years follow-up

	Retinal microvascular measures	Cognitive outcome measures	Imaging outcome measures	Additional adjustments[b]
	Retinopathy, focal narrowing, arteriovenous nicking	N/A	Microbleeds	Vascular risk factors[c], brain imaging markers[d]
		N/A	Cerebral infarcts (cortical and subcortical), white matter lesions	Vascular risk factors, brain imaging markers
		Dementia, memory, processing speed, executive functioning and attention	N/A	Vascular risk factors, brain imaging markers, vision[e], depressive symptoms
	Retinopathy, focal narrowing, arteriovenous nicking, arteriolar narrowing	Any cognitive impairment no dementia, memory, processing speed, executive functioning and attention	N/A	Vascular risk factors, alcohol
		N/A	White matter lesions	Vascular risk factors
		N/A	Atrophy (subcortical and cortical)	Vascular risk factors
		N/A	Cerebral infarcts (lacunar and non-lacunar)	Vascular risk factors
		Rapid decline in memory, processing speed, executive functioning and dementia	N/A	Vascular risk factors, ApoE, alcohol
		N/A	Atrophy (subcortical and cortical)	Vascular risk factors
	Retinopathy, focal narrowing, arteriovenous nicking	N/A	Cerebral infarcts (lacunar and non-lacunar), white matter lesions	Vascular risk factors

Table 1. continued

Study cohort	Report	N (% of eligible)	Age[a]	Male	Design
Blue Mountains Eye Study (BMES),	[14]	1988 (85%)	69	43%	Cross-sectional
Cardiovascular Health Study (CHS)	[15]	2211 (65%)	78	40%	Cross-sectional
	[33]	3660	78	40%	Cross-sectional
	[33]	2116	78	40%	5 years follow-up
	[16]	1744 (31%)	78	40%	Cross-sectional
Los Angeles Latino Eye Study (LALES) Latino adults: high diabetes and hypertension prevalence	[17]	809 (28%)	70	41%	Cross-sectional
Lothian Birth Cohort 1921	[18]	321 (74%)	83	45%	Cross-sectional
Rotterdam Study	[34]	490	68	51%	Cross-sectional

Retinal microvascular measures	Cognitive outcome measures	Imaging outcome measures	Additional adjustments[b]
Retinopathy, arteriolar narrowing, venular dilation	Any cognitive impairment no dementia	N/A	Vascular risk factors
Retinopathy, focal narrowing, arteriovenous nicking, arteriolar narrowing, venular dilation	Dementia, mental state / dementia screening, processing speed	N/A	Vascular risk factors, brain imaging markers
	N/A	Cerebral infarcts, white matter lesions	None
	N/A	Cerebral infarcts, white matter lesions	None
	Processing speed	N/A	Vascular risk factors, alcohol
Retinopathy, arteriolar narrowing, venular dilation	Mental state / dementia screening, memory, executive functioning and attention, fluid intelligence	N/A	Vascular risk factors, depression
Arteriolar narrowing, venular dilation	Mental state / dementia screening, memory, executive functioning and attention, fluid intelligence	N/A	Vascular risk factors, vision, ApoE, alcohol
Arteriolar narrowing, venular dilation	N/A	Cerebral infarcts (lacunar and non-lacunar), white matter lesions	Vascular risk factors

Table 1. continued

Study cohort	Report	N (% of eligible)	Age[a]	Male	Design
Rotterdam Study (contined)	[34]	279	68	51%	3.3 years follow-up
	[19]	5553 (82%; baseline)	68	41%	11.6 years follow-up
	[20]	6273 (61%)	69	41%	Cross-sectional and 11.4 years follow-up
Singapore Malay Eye Study (SiMES)	[21]	1179 (55%)	69	53%	Cross-sectional
	[22]	1202 (29%)	69	52%	Cross-sectional
Women's Health Initiative (WHI) Postmenopausal women	[23]	505[f]	69	0%	10 years follow-up (cognitive screening)

Retinal microvascular measures	Cognitive outcome measures	Imaging outcome measures	Additional adjustments[b]
	N/A		Vascular risk factors
Arteriolar narrowing, venular dilation	Dementia	N/A	Vascular risk factors
Retinopathy	Dementia	N/A	Vascular risk factors, ApoE
Retinopathy	Any cognitive impairment no dementia	N/A	Vascular risk factors, vision
Retinopathy, arteriovenous nicking, focal narrowing, arteriolar narrowing, venular dilation	Any cognitive impairment no dementia	N/A	Vascular risk factors
Retinopathy	Mental state / dementia screening	White matter lesions, lacunar infarcts, atrophy	Vascular risk factors, brain imaging markers

Table 1. continued

Study cohort	Report	N (% of eligible)	Age[a]	Male	Design
Patient-based					
Edinburgh Type 2 Diabetes Study (ET2DM) (Type 2 diabetes)	[24]	1044 (98%)	67	51%	Cross-sectional
	[25]	954 (89%)	67	51%	Cross-sectional
Japanese Elderly Diabetes Intervention Trial (J-EDIT) (Type 2 diabetes)	[27]	907 (77%)	72	45%	Cross-sectional
Utrecht Diabetic Encephalopathy Study (UDES) (Type 2 diabetes)	[35]	122[f]	66	51%	Cross-sectional
	[26]	68[f]	66	48%	Cross-sectional and 4 years follow-up
Seoul National University Hospital (Hypertension)	[36]	550[f]	59	68%	Cross-sectional
Multi-Center Retina and Stroke Study (MCRS) (Acute stroke)	[37]	1360[f]	66	61%	Cross-sectional

	Retinal microvascular measures	Cognitive outcome measures	Imaging outcome measures	Additional adjustments[b]
	Retinopathy	Mental state / dementia screening, memory, processing speed, executive functioning and attention, fluid intelligence	N/A	Vascular risk factors, depressive symptoms
	Arteriolar narrowing, venular dilation		N/A	Vascular risk factors, depressive symptoms
	Retinopathy	Any cognitive impairment no dementia	N/A	None
	Retinopathy	N/A	Atrophy (cortical and subcortical)	None
	Retinopathy	Memory, processing speed, executive functioning and attention, fluid intelligence	White matter lesions, atrophy (cortical and subcortical)	None
	Retinopathy	N/A	Lacunar infarcts	Vascular risk factors
	Retinopathy, arteriovenous nicking, focal narrowing, arteriolar narrowing, venular dilation	N/A	Atrophy (cortical and subcortical)	Vascular risk factors

Table 1. continued

Study cohort	Report	N (% of eligible)	Age[a]	Male	Design
Amsterdam Vascular Medicine Group (Symptomatic atherosclerotic disease)	38	358[f]	62	67%	Cross-sectional

ApoE, apolipoprotein E status.

[a] For longitudinal studies age at baseline.

[b] All studies adjusted their analyses for at least one of the following demographics: age, sex, education, ethnicity, center, pre-morbid IQ, socio-economic status, income, housing, or occupation.

[c] Vascular risk factors included type 2 diabetes mellitus, macrovascular disease, myocardial infarction, history of cardiovascular disease, hypertension, systolic blood pressure, diastolic blood pressure, mean arterial pressure, antihypertensive medication, anticoagulants use, intima-media thickness, glucose, HbA1c, cholesterol, triglycerides, lipid-lowering medication, C-reactive protein, body mass index, waist-to-hip ratio, smoking.

[d] Brain imaging markers included brain infarcts, white matter lesions, cerebral microbleeds, ischemic lesion volumes.

[e] Vision included visual acuity, cataract, age-related macula degeneration, glaucoma, concurrent eye diseases.

[f] Percentage of eligible participants unknown.

Retinal microvascular measures	Cognitive outcome measures	Imaging outcome measures	Additional adjustments[b]
Retinopathy, arteriolar narrowing	N/A	White matter lesions, lacunar infarcts	Vascular risk factors

Brain imaging measures

Studies on five population-based cohorts (AGES,[28] ARIC,[4,29–32] CHS,[33] Rotterdam Study,[34] WHI[23]) and four patient-based cohorts (patients with diabetes J-EDIT,[27] UDES,[26,35] hypertension,[36] acute stroke,[37] or symptomatic atherosclerosis[38]) reported on retinal microvascular changes and brain imaging measures. All studies used magnetic resonance imaging (MRI) scans, one study used computed tomography (CT) scans in a subgroup of participants.[38] Reported brain imaging abnormalities were infarcts, white matter lesions, and atrophy. In some studies these were subdivided by type (whole brain or lacunar infarcts) or by location (subcortical or periventricular white matter lesions; subcortical or cortical atrophy). All population-based studies assessed cerebral infarcts and white matter lesions; in two population-based and two patient-based cohorts atrophy was reported.

Several definitions for cerebral infarcts were used, based on lesion size, location, and appearance on different MRI sequences.[4,11,28,33,36,51] In Rotterdam[51] and ARIC,[4,32] a subset of infarcts was classified as lacunar if they had a diameter <20 mm and were located in the basal ganglia, internal capsule, thalamus, or deep cerebral white matter. In AGES[11] infarcts were subdivided into cortical and subcortical (i.e. lacunar) infarcts, whereas in CHS[33] no distinction in types of infarcts was made. In WHI[23] lacunar infarctions were defined as ischemic lesions located in basal ganglia, as determined by automatic segmentation.

White matter lesions were defined as hyperintense lesions on T2-weighted and FLAIR images,[11,23,26,35] on T2-weighted and proton-density images without prominent hypo-intensity on T1-weighted images,[34] or as white matter signal abnormality on spin-density images;[29] two studies did not specify MRI characteristics of white matter lesions.[33,38] In WHI[23] and UDES,[26] ischemic white matter disease volume was determined by automatic segmentation, whereas AGES, ARIC, CHS, Rotterdam used an ordinal scale for white matter lesion grading. Two studies[19,20,28] subdivided white matter lesions into periventricular or subcortical.

For assessing atrophy, ARIC investigators[30,31] used a visual grading scale for sulcal widening and ventricular enlargement on T1-, T2-, and proton density-weighted images, independently of focal abnormalities and white matter lesions. One study in hypertensive patients used an atrophy grading

scale for CT and MRI scans.[37] In two studies[23,26] T1-weighted volumetric MRI scans were automatically segmented and regional brain volumes were calculated.

Only one study examined microbleeds,[39] defined as a focal area of signal void within the brain parenchyma.

In three population-based cohorts and one cohort of patients with diabetes, changes in brain imaging abnormalities over time were examined (ARIC,[31,32] CHS,[33] Rotterdam,[34] and UDES.[26]) Incidence of infarcts were measured, as well as changes in white matter lesions defined as progression on a ordinal grading scale. Progression of atrophy was measured in the ARIC study, where it was defined as an increase in ventricular size and sulcal size on a visual ordinal grading scale, and in the UDES study where brain volume changes were calculated.

Associations between retinal microvascular changes and dementia or cognition

Cross-sectional studies: any retinopathy

Nine population-based studies reported on the relation between any retinopathy and cognitive outcomes. Six studies reported on a clinical diagnosis of dementia or impaired cognitive functioning (see Table 2a), all of which found an increased risk (range ORs 1.17 to 5.57), which was statistically significant in two studies. Four studies reported on the relation between any retinopathy and cognitive performance as a continuous measure. These studies found that overall, persons with any retinopathy tended to perform worse on the cognitive tasks, for all cognitive domains. However, effect sizes were small (range -0.25 to 0.03, Table 2a) and associations were statistically significant in only one study.

Pooled random effect models showed that across studies, any retinopathy was significantly associated with presence of dementia (pooled OR 1.51, 95% confidence interval (CI) 1.14 to 1.99) and cognitive impairment (pooled OR 1.97, 95% CI 1.27 to 3.06), as well as with decreased processing speed (pooled effect size -0.07, 95% CI -0.11 to -0.03).

In eight studies the modulating role of vascular risk factors was explored by adjusting for a range of factors or by stratifying for hypertension and diabetes. In two studies, the relation between any retinopathy and dementia[15] or

cognitive impairment[14] was stronger in participants with hypertension than in those without. One study only found a statistically significant relation between any retinopathy and dementia in persons without diabetes.[15] The highest risk of dementia or cognitive impairment was found in a population of Latinos with a high diabetes and hypertension prevalence.[17] Three studies found no differences according to hypertension or diabetes status.[12,20] All three studies that examined dementia found stronger results for vascular dementia or mixed pathology than for "pure" Alzheimer's disease.[11,15,20]

Regarding patient-based cohorts, studies in patients with type 2 diabetes observed associations within the range of the population-based studies (Table 2b). One study in patients with diabetes found no association between retinopathy and cognitive functioning at baseline or cognitive decline after four years.[26,35]

Cross-sectional studies: focal narrowing, arteriovenous nicking or retinal vascular caliber

Eight studies describing seven different population-based cohorts reported on the relation between focal narrowing, arteriovenous nicking, or vessel caliber (i.e. generalized arteriolar narrowing or generalized venular widening) and dementia or cognitive functioning. For all measures an increased risk of dementia or cognitive impairment was found, but ORs were lower compared with any retinopathy (range 1.08 to 2.04, Table 2a). Associations between focal vascular lesions or vascular caliber and domains of cognitive functioning were found in both directions and were mostly not statistically significant. In one study suboptimal retinal vascular networks, but not focal retinal lesions or retinal caliber, were significantly associated with cognitive functioning.[22]

Stratifying for hypertension or diabetes status revealed in one study that the association between retinal caliber and cognitive impairment was driven by persons with hypertension.[17] Another study found that focal narrowing was associated with dementia only in persons with diabetes.[15] Patient-based data were available of one study, the authors found no relation between retinal caliber and cognitive functioning[25] (Table 2b).

Longitudinal studies

In three population-based cohorts retinal microvascular changes in relation

to incident dementia or to accelerated cognitive decline ten to fourteen years later were examined (see Table 2c). Any retinopathy was not related to risk of dementia (Rotterdam study[20]), but associations were found with an increased risk of decline in processing speed and executive functioning and attention (OR 2.18 and 1.33, respectively) (ARIC[13]), and with poorer performance on a cognitive screening instrument (3MSE) throughout follow-up (WHI[23]). Focal narrowing was associated with a decline in executive functioning (ARIC[13]). Arteriolar narrowing was unrelated to dementia risk or cognitive decline.[13,19] Venular widening was associated with incident dementia in the Rotterdam study (HR 1.11),[19] but unrelated to cognitive decline in ARIC.[13] One study in patients with diabetes found no association between diabetic retinopathy and cognitive change after four years.[26]

Two studies with longitudinal data adjusted or stratified for vascular risk factors; the results did not change.[13,20]

Associations between retinal microvascular changes and brain imaging

Cross-sectional studies: any retinopathy

Three population-based studies reported on the relation between any retinopathy and brain imaging abnormalities. Only ARIC[31,32] found significant associations for infarcts, white matter lesions and atrophy (ORs from all studies ranged from 0.94 to 2.95, Table 2a). One study found an association between retinopathy and multiple microbleeds (OR 1.75; 95% CI 1.25 to 2.45).[39]

After correction for vascular risk factors, associations with all brain imaging outcomes attenuated slightly. Stratifying for hypertension or diabetes status showed that, overall, associations between any retinopathy and imaging abnormalities were stronger in persons with hypertension or diabetes than in those without.

In patient-based studies (see Table 2b), retinopathy was associated with infarcts in a cohort of patients with hypertension;[36] with lacunar infarcts and white matter lesions in a cohort of patients with symptomatic atherosclerotic disease;[38] with cortical atrophy in a cohort of patients with diabetes;[26,35] and with subcortical atrophy in a cohort of patients with acute stroke[15] (ORs within range of population-based results).

Table 2a. Associations between retinal microvascular changes and outcome measures of cognitive functioning and MRI abnormalities in cross-sectional population-based studies

Study cohort	Cognitive outcome measures					
	Dementia	**Cognitive impairment (no dementia)**	**Mental state**	**Memory**	**Processing speed**	**EF&A**
	OR (95% CI)[a]		**Effect size[a,b]**			
Any retinopathy *Retinal measure dichotomized: retinopathy present versus absent*						
AGES[11,28]	1.35 (0.89;2.04)	..	..	-0.04	-0.05	-0.02
ARIC[4,12,29,30,32]	..	**2.03* (1.07;3.86)**	..	**-0.25***	**-0.11***	**-0.11***
Blue Mountains Eye[14]	..	No hypertension 0.5(0.1;1.6) **Hypertension 1.7*(1.0;3.2)**	..	..	..	..
CHS[15,16,33]	1.17 (0.62;2.22)	..	-	..	-0.04	..
LALES[17]	..	1.23 (0.42;3.54)	-0.11	0.03	..	-0.02
Rotterdam[19,20]	**1.92* (1.24;2.98)**	..	..	..	..	..
SiMES[21]	..	**5.57* (1.56;19.91)**	..	..	..	..
Pooled random effects[c]	**1.51* (1.14;1.99)**	**1.97* (1.27;3.06)**	..	-0.09 (-0.25; 0.07)	**-0.07* (-0.11; -0.03)**	-0.06 (-0.14; 0.02)

	Brain imaging outcome measures					
Fluid intelligence	**Any infarct**	**Lacunar infarct**	**Subcortical WML**	**Periventricular WML**	**Subcortical atrophy**	**Cortical atrophy**
Effect size[a,b]	**OR (95% CI) or direction of association**					
..	1.06 (0.89;1.26)	1.10 (0.86;1.40)	1.11 (0.88;1.41)	1.12 (0.91;1.37)	..	..
..	**2.95* (1.30;6.59)**	..	**2.50* (1.50;4.00)**		**1.50* (1.00;2.30)**	**1.90* (1.20;3.00)**
..	..	..	..	..	..	..
..	0.94 (0.63;1.41)	..	+	..	..	..
..	..	..	..	..	..	..
..	..	..	..	..	..	..
..	..	..	..	..	..	..
..	..[d]	..	..	..	..	..

Table 2a. continued

Study cohort	Cognitive outcome measures					
	Dementia	Cognitive impairment (no dementia)	Mental state	Memory	Processing speed	EF&A
	OR (95% CI)[a]		Effect size[a,b]			
Arteriovenous nicking *Retinal measure dichotomized: arteriovenous nicking present versus absent*						
AGES[11]	=	..	..	=	=	=
ARIC[4,12,29–32]	..	1.15 (0.81;1.62)	..	-0.01	-0.03	**-0.08***
CHS[15,16,33]	1.48 (0.74;2.96)	..	=	..	**-0.04***	..
SiMES[22]	..	=	..	..	..	..
Focal narrowing *Retinal measure dichotomized: focal narrowing present versus absent*						
AGES[11]	=	..	..	=	=	=
ARIC[4,12,29–32]	..	1.24 (0.76;2.03)	..	**0.07***	0.04	0.01
CHS[15,16,33]	**1.99* (1.11;3.56)**	..	-	..	-0.02	..
SiMES[22]	..	=	..	..	..	..
Arteriolar narrowing *Retinal measure dichotomized: generalized arteriolar narrowing defined as the narrowest decile, quintile or quartile*						
ARIC[4,12,29–32]	..	1.10 (0.80;1.49)	..	-0.03	-0.02	-0.01
Blue Mountains Eye[14]	..	No hypertension 0.4 (0.10;1.30) Hypertension 2.2 (0.90;5.20)	..	..	..	..

	Brain imaging outcome measures					
Fluid intelligence	**Any infarct**	**Lacunar infarct**	**Subcortical WML**	**Periventricular WML**	**Subcortical atrophy**	**Cortical atrophy**
	OR (95% CI) or direction of association					
..	1.07 (0.93;1.22)	**1.33* (1.09;1.62)**	**1.64* (1.36;1.97)**	**1.54* (1.31;1.81)**	..	..
..	**1.90* (1.25;2.88)**	..	**2.10* (1.40;3.20)**	1.20 (0.80;1.60)	1.20 (0.90;1.70)	
..	**1.84* (1.23;2.76)**	..	-		..	..
..	..	..	..	..	..	..
..	1.08 (0.90;1.29)	1.19 (0.92;1.54)	**1.40* (1.09;1.79)**	**1.40* (1.12;1.74)**	..	..
..	**1.89* (1.22;2.92)**	..	**2.10* (1.40;3.10)**	1.20 (0.90;1.70)	1.10 (0.80;1.60)	..
..	1.20 (0.81;1.78)	..	-	..	..	..
..	..	..	..	..	..	..
..	1.74 (0.95;3.21)	..	1.20 (0.80;1.90)		1.10 (0.80;1.50)	1.00 (0.70;1.40)
..	..	..	..	..	..	..

Table 2a. continued

Study cohort	Cognitive outcome measures					
	Dementia	**Cognitive impairment (no dementia)**	**Mental state**	**Memory**	**Processing speed**	**EF&A**
	OR (95% CI)[a]					**Effect size[a,b]**
Arteriovenous nicking						
Retinal measure dichotomized: arteriovenous nicking present versus absent						
CHS[15,16,33]	1.42 (0.74;2.73)	..	=	..	**-0.03***	..
LALES[17]	..	**2.04* (1.14;3.66)**	-0.30	0.06	..	-0.15
Retinal measure as a continuous variable: arteriolar caliber[c]						
Lothian Birth[18]	..	..	=	=	..	=
Rotterdam[34]	..	..	..	..	..	..
SiMES[22]	..	=	..	..	..	..
Venular dilation						
Retinal measure dichotomized: venular dilation defined as the widest decile, quintile or quartile						
Blue Mountains Eye[14]	..	No hypertension 1.00(0.40;2.40) **Hypertension 2.7*(1.2;6.1)**	..	..	..	..
CHS[15,33]	..	..	=	..	0.03	..
LALES[17]	..	1.08 (0.59;1.96)	-0.50	-0.14	..	0.23
Retinal measure as a continuous variable: venular caliber						
Lothian Birth[18]	..	..	=	=	..	=
Rotterdam[34]	..	..	..	..	..	..
SiMES[22]	..	=	..	..	..	..

EF&A, Executive functioning and attention; WML, white matter lesions;

 OR, odds ratio; CI, confidence interval.

	Brain imaging outcome measures					
Fluid intelligence	**Any infarct**	**Lacunar infarct**	**Subcortical WML**	**Periventricular WML**	**Subcortical atrophy**	**Cortical atrophy**
	OR (95% CI) or direction of association					
..	1.15 (0.99;1.26)	..		-	..	..
..	..	..	..	..	..	..
=	..	..	..	..	..	..
..	..	1.14 (0.91;1.44)	=	=	..	..
..	..	..	..	..	..	..
..	..	..	..	..	..	..
..	1.05 (0.93;1.19)	..		-	..	..
..	..	..	..	..	..	..
=	..	..	..	..	..	..
..	..	1.07 (0.85;1.35)	=	=	..	..
..	..	..	..	..	..	..

*p<0.05; . ., outcome measure not evaluated; –, outcome measure evaluated, presence of retinal microvascular changes associated with worse cognition or more white matter lesions; +, outcome measure evaluated, presence of retinal microvascular changes associated with better cognition or less white matter lesions; =, no association between presence of retinal microvascular changes and outcome measure.

[a] Median ORs or effect sizes are presented in case multiple results were reported for the same measure.

[b] Expressed as Cohen's d or standardized regression coefficients

[c] Data were pooled for studies investigating any retinopathy and cognitive outcome measures, because data were available from multiple studies that were similar in design, weighted ORs or effect sizes are presented for fully adjusted data.

[d] Studies investigating any retinopathy in relation to infarcts were too heterogeneous (I^2 69%, p=0.04).

[e] Results for arteriolar caliber were recoded in such a way that a negative coefficient means arteriolar narrowing is associated with worse cognition or more brain imaging abnormalities.

Table 2b. Associations between retinal microvascular changes and outcome measures of cognitive functioning in patient-based studies

Study cohort	**Cognitive outcome measures**					
	Dementia	**Cognitive impairment (no dementia)**	**Mental state**	**Memory**	**Processing speed**	**EF&A**
	OR (95% CI)[a]		**Effect size[a]**			
Any retinopathy *Retinal measure dichotomized: retinopathy present versus absent*						
Edinburgh[25] **Diabetes**	..	..	**-0.02***	-0.01	**-0.03***	**-0.02***
J-EDIT[27] **Diabetes**	..	1.73 (1.00;3.00)	..	..	..	..
UDES[26,35] **Diabetes**	..	..	=	=	=	=
Seoul[36] **Hypertension**	..	..	..	..	..	..
MCRS[37] **Acute stroke**	..	..	..	..	..	..
Amsterdam Vascular Medicine Group[38] **Symptomatic atherosclerotic disease**	..	..	..	..	..	..
Arteriovenous nicking *Retinal measure dichotomized: arteriovenous nicking present versus absent*						
MCRS[37] **Acute stroke**	..	..	..	..	..	..
Focal narrowing *Retinal measure dichotomized: focal narrowing present versus absent*						
MCRS[37] **Acute stroke**	..	..	..	..	..	..

	Brain imaging outcome measures					
Fluid intelligence	**Any infarct**	**Lacunar infarct**	**Subcortical WML**	**Periventricular WML**	**Subcortical I atrophy**	**Cortical atrophy**
	OR (95% CI) or direction of association					
0.00	..	..	..	..	..	..
..	..	..	..	..	..	..
=	..	..	=	=	=	-*
..	**2.98* (1.20;7.42)**	..	..	..	..	..
..	..	..	..	..	**1.90* (1.20;3.00)**	0.90 (0.60;1.30)
..	..	(combined for lacunar infarcts and white matter lesions)	-*		..	..
..	..	..	..	..	1.40 (0.90;2.30)	1.00 (0.60;1.50)
..	..	..	..	..	0.80 (0.40;1.60)	0.70 (0.40;1.30)

Table 2b. continued

Study cohort	Cognitive outcome measures					
	Dementia	**Cognitive impairment (no dementia)**	**Mental state**	**Memory**	**Processing speed**	**EF&A**
	OR (95% CI)[a]					**Effect size[a]**
Arteriolar narrowing *Retinal measure dichotomized: arteriolar narrowing defined as the narrowest quintile*						
MCRS[37] **Acute stroke**	..	..	..	..	..	..
Amsterdam Vascular Medicine Group[38] **Symptomatic atherosclerotic disease**	..	..	..	..	..	..
Retinal measure as a continuous variable: arteriolar caliber[b]						
Edinburgh[25] **Diabetes**	..	..	..	0.01	0.03	0.00
Venular dilation *Retinal measure dichotomized: venular dilation defined as the widest quintile*						
MCRS[37] **Acute stroke**	..	..	..	..	..	..
Retinal measure as a continuous variable: venular caliber[1]						
Edinburgh[25] **Diabetes**	..	..	..	0.00	0.02	0.00

EF&A, Executive functioning and attention; WML, white matter lesions; OR, odds ratio; CI, confidence interval.

*p<0.05; . ., outcome measure not evaluated; –, outcome measure evaluated, presence of retinal microvascular changes associated with worse cognition or more white matter lesions; +, outcome measure evaluated, presence of retinal microvascular changes associated with better cognition or less white matter lesions; =, no association between presence of retinal microvascular changes and outcome measure.

	Brain imaging outcome measures						
Fluid intelligence	**Any infarct**	**Lacunar infarct**	**Subcortical WML**	**Periven-tricular WML**	**Subcortical I atrophy**	**Cortical atrophy**
	OR (95% CI) or direction of association					
..	..	..	..	..	1.00 (0.60;1.70)	0.90 (0.50;1.50)
..	..	-* (combined for lacunar infarcts and white matter lesions)			..	..
-0.01	..	..	..	..	..	..
..	..	..	..	..	1.30 (0.80;2.20)	0.90 (0.60;1.50)
0.02	..	..	..	..	..	..

[a] Expressed as Cohen's d or standardized regression coefficients

[b] Results for arteriolar caliber were recoded in such a way that a negative coefficient means arteriolar narrowing is associated with worse cognition.

Table 2c. Associations between retinal microvascular changes and outcome measures of cognitive functioning and MRI abnormalities in longitudinal population-based studies

Study cohort	Cognitive outcome measures					
	Dementia	**Cognitive impairment (no dementia)**	**Mental state**	**Memory**	**Processing speed**	**EF&A**
	HR (95% CI)	**OR (95% CI)**	**Effect size[a]**			
Any retinopathy *Retinal measure dichotomized: retinopathy present versus absent*						
ARIC[13,31,32]	..	1.33 (0.41;2.52)	..	+	+	-*
Rotterdam[20]	1.15 (0.89;1.50)	..	..	..	..	..
WHI[23]	..	..	-*	..	..	..
Arteriovenous nicking *Retinal measure dichotomized: arteriovenous nicking present versus absent*						
ARIC[13,31,32]	..	0.77 (0.37;1.61)	..	-	-	-
Focal narrowing *Retinal measure dichotomized: focal narrowing present versus absent*						
ARIC[13,31,32]	..	1.55 (0.85;2.83)	..	-	+	-*
Arteriolar narrowing *Retinal measure dichotomized: generalized arteriolar narrowing defined as the narrowest quartile*						
ARIC[13,31,32]	..	0.95 (0.54;1.70)	..	+	+	-
Retinal measure as a continuous variable: arteriolar caliber[b]						
ARIC[13]	..	..	..	=	=	=
Rotterdam[19,34]	1.05 (0.96;1.16)	..	..	..	..	..

	Brain imaging outcome measures					
Fluid intelligence	**Any infarct**	**Lacunar infarct**	**Subcortical WML**	**Periven-tricular WML**	**Subcortical I atrophy**	**Cortical atrophy**
	OR (95% CI) or direction of association					
..	**2.82*** **(1.42;5.60)**	**3.19*** **(1.56;6.50)**	1.54 (0.65;3.61)		**2.03*** **(1.02;4.42)**	1.03 (0.37;2.90)
..	..	..	..	..	**..**	**..**
..	**-***	**-***	**-***		**=**	**=**
..	**2.82*** **(1.66;4.76)**	**2.48*** **(1.39;4.40)**	**2.12*** **(1.18;3.81)**		**2.19*** **(1.23;3.90)**	1.37 (0.67;2.81)
..	1.02 (0.58;1.78)	**1.13** **(0.62;2.07)**	**0.89** **(0.46;1.67)**		**1.13** **(0.61;2.10)**	**1.30** **(0.66;2.55)**
..	..	..	..	..	..	..
..	..	..	..	..	=	=
..	..	0.81 (0.54;1.22)	0.85 (0.65;1.11)	0.93 (0.70;1.22)	..	..

Table 2c. continued

Study cohort	Cognitive outcome measures					
	Dementia	**Cognitive impairment (no dementia)**	**Mental state**	**Memory**	**Processing speed**	**EF&A**
	OR (95% CI)[a]		**Effect size[a]**			
Venular dilation						
Retinal measure dichotomized: venular dilation defined as the widest quartile						
ARIC[13,31,32]	..	..	..	=	=	=
Rotterdam[19,34]	**1.11* (1.00;1.22)**	..	..	..	..	..
Retinal measure as a continuous variable: venular caliber						
ARIC[13,31,32]	..	..	..	=	=	=

EF&A, Executive functioning and attention; WML, white matter lesions; HR, hazard ratio; OR, odds ratio; CI, confidence interval.

*p<0.05; . ., outcome measure not evaluated; –, outcome measure evaluated, presence of retinal microvascular changes associated with worse cognition or more white matter lesions; +, outcome measure evaluated, presence of retinal microvascular changes associated with better cognition or less white matter lesions; =, no association between presence of retinal microvascular changes and outcome measure.

[a] Expressed as Cohen's d or standardized regression coefficients

[b] Results for arteriolar caliber were recoded in such a way that a negative coefficient means arteriolar narrowing is associated with worse cognition.

	Brain imaging outcome measures						
Fluid intelligence	**Any infarct**	**Lacunar infarct**	**Subcortical WML**	**Periven-tricular WML**	**Subcortical I atrophy**	**Cortical atrophy**
	OR (95% CI) or direction of association					
..	..	..	..	..	..	..
..	..	1.24 (0.72;2.12)	**2.50* (1.30;4.81)**	**1.74* (1.02;2.95)**	..	..
..	..	..	..	..	=	=

Cross-sectional studies: focal narrowing, arteriovenous nicking or retinal vascular caliber

Focal narrowing and arteriovenous nicking were significantly associated with brain imaging abnormalities in three out of three population-based cohorts for infarcts (whole brain or lacunar) and white matter lesions (subcortical or periventricular), ORs ranged from 1.07 to 2.10 (Table 2a). Associations with atrophy were not significant. One study found an association between focal narrowing and multiple microbleeds (OR 1.45; CI 1.01 to 2.09), and between arteriovenous nicking and multiple microbleeds (OR 1.44; CI 1.06 to 1.95).[39]

Associations of arteriolar narrowing and venular dilation with imaging abnormalities were generally weaker compared with the other retinal measures. In three out of three population-based cohorts, effects were very small and not significant, for all imaging abnormalities (OR range 1.05 to 1.74).

As with any retinopathy, associations with brain imaging outcomes attenuated slightly after correction for vascular risk factors. In a cohort of patients with symptomatic atherosclerotic disease, arteriolar narrowing was associated with lacunar infarcts and white matter lesions.[38] No significant associations with atrophy were found in acute stroke patients (ORs range 0.70 to 1.40, Table 2b).

Longitudinal studies

In three population-based cohorts retinal microvascular changes were examined in relation to progression of brain imaging abnormalities three to ten years later. Any retinopathy at baseline was related to an increased risk of (lacunar) infarcts, white matter lesions or subcortical atrophy at follow-up, in one or two of two study cohorts (median OR 2.03; Table 2c). Focal narrowing and arteriovenous nicking were examined only in ARIC. Arteriovenous nicking was significantly related to incident (lacunar) infarcts, white matter lesions or subcortical atrophy (median OR 2.19), whereas focal narrowing was unrelated to progression of brain imaging abnormalities (median OR 1.13). Regarding measures of caliber, only venular dilation was related to white matter lesion progression, and, in unadjusted analyses only, to lacunar infarcts (arteriolar narrowing: median OR 0.85; venular dilation: median OR 1.74). For none of the retinal microvascular changes a relation with cortical atrophy after follow-up was found.

In a cohort of patients with diabetes, no associations between retinopathy

and brain imaging changes after follow-up were found.[26,35]

Four longitudinal studies adjusted or stratified for vascular risk factors and found varying results. After adjusting, the relation attenuated in some cases (between venular widening and incident infarcts[34]), became stronger in others (between retinopathy and arteriovenous nicking and subcortical atrophy[31]), or did not change.[23,31] One study found that any retinopathy was more strongly associated with incident infarcts and white matter lesion progression 10 years later in participants with diabetes and/or without hypertension, whereas for arteriovenous nicking this was the case in participants without diabetes and with hypertension.[32]

DISCUSSION

The relations between microvascular changes in the retina and dementia or cognitive functioning and between these microvascular changes and brain imaging abnormalities have been examined in several large cohort studies in the last decade. Despite considerable heterogeneity in measures of both retinal microvascular changes and the cerebral outcomes, cross-sectional studies found consistent and moderately strong associations with dementia, with modest decrements in cognitive functioning in non-demented people, as well as with brain imaging abnormalities. This supports a role for vascular disease as the underlying pathophysiology of cognitive decline and brain changes. The few available longitudinal studies found associations with progression of brain imaging abnormalities, but only marginally with incident dementia or cognitive decline.

In the light of the results of this systematic review, some methodological issues warrant consideration. First, a formal meta-analysis was not performed, because of the heterogeneity of study designs and outcome measures. Second, studies with negative results may have been underrepresented due to the effects of publication bias. Third, no information was available on the cognitive domains language or perception, because these were tested in none of the studies.

Moreover, compared with the cross-sectional studies, longitudinal studies were fewer in number and tended to have lower power to find associations between the retinal microvascular changes and the cerebral outcomes.

Nevertheless, in longitudinal studies somewhat weaker associations were found than in cross-sectional studies, especially with regard to dementia and cognitive decline. If this is a real effect, it might be caused by competing risk effects of mortality. It is also conceivable that retinal vascular pathology occurs at the same time as clinical symptoms of dementia or cognitive impairment and is a relatively late phenomenon in the etiological cascade.[20] At present, in our view, no definite conclusions with regard to the prospective associations between retinal microvascular changes and the cerebral outcomes should be drawn and a key target for future studies is to obtain reliable longitudinal data from large datasets.

The reported association between retinal vascular changes and adverse cerebral outcomes conveys an important etiological message. Abnormalities in the retinal vasculature can be considered a marker of a disadvantageous vascular risk factor profile and of an individual's vulnerability to adverse outcomes. Indeed, the measures of the retinal vessels that are described in this review reflect increasing exposure to high blood pressure. Arteriolar narrowing and venular dilation are early consequences of a rise in blood pressure. Focal narrowing and arteriovenous nicking develop later, in the phase of chronic hypertension. When blood pressure rises further and requires acute treatment, the blood-retinal barrier breaks down and signs of retinopathy occur, such as hemorrhages, hard exudates and cotton-wool spots.[5,34] A similar gradient in severity of pathologies is reflected in associations between retinal vascular changes and systemic vascular disease. Milder retinal vascular changes such as generalized arteriolar narrowing, focal narrowing, and arteriovenous nicking are weakly related to an increased risk of cerebrovascular and cardiovascular disease, whereas this relation is stronger for moderately severe forms such as retinopathy signs.[52–54] These findings corroborate the results of this review in that the strength of the association with cognition and brain imaging abnormalities increases as the vascular disease process progresses: associations with adverse outcomes were relatively weak for vascular caliber measurements, intermediate for focal narrowing and arteriovenous nicking, and stronger for any retinopathy. The role of vascular disease is also illustrated by the fact that associations with cognitive impairment and brain infarcts were stronger in persons with hypertension. A similar finding is reported for diabetes mellitus:

in persons with diabetes, retinopathy signs were more strongly associated with adverse outcomes than in persons without diabetes.

Another finding of etiological significance is the fact that retinal vascular changes are associated with multiple types of brain imaging abnormalities, including atrophy, several manifestations of small vessel disease, as well as large vessel infarcts. This may reflect the fact that retinal and cerebral pathologies share common risk factors and etiologies. It is also possible that retinal vascular changes and brain disease are the result of several etiological pathways developing simultaneously in the context of aging and an adverse vascular risk factor profile.

The prognostic value of a risk marker depends on its prevalence and on the strength of the association with the outcomes. Associations found for any retinopathy, which reflects moderately severe microvascular disease, were strongest, but the prevalence of retinopathy is only 2-15% in the general population.[55] Changes in retinal vascular caliber are milder and more common, but were only weakly related to cerebral outcomes. Consequently, only a small proportion of cases of dementia, cognitive impairment, or brain changes in the population is attributable to variations in retinal vasculature. Therefore, at the population level, retinal vascular assessment is not considered to be a useful predictor for adverse cerebral outcomes by itself. At best it can be incorporated in prediction models together with other predictors, although there is doubt whether this can provide vital prognostic information in addition to traditional risk factors.[56] Nevertheless, presence of more severe retinal microvascular abnormalities can still be used to identify individuals at elevated risk.

Some contrasts in strengths of associations deserve attention because they provide insight in the nature of the relation between retinal microvascular changes and the outcomes. First, stronger cross-sectional associations were found in the middle-aged ARIC population (mean baseline ages ranging from 54 to 62 years), than in other populations with an age range from 69 to 83 years. These findings are in line with an age-related decrease in the strength of associations that have been found for vascular risk factors and blood pressure[57] and cerebrovascular and cardiovascular disease[53] in relation to retinopathy signs, as well as for vascular risk factors such as hypertension, diabetes, dyslipidemia, and obesity in relation to dementia.[58] Second, associations with retinal microvascular changes were

stronger for dementia and other definitions of severe cognitive impairment, than for milder forms of cognitive dysfunction. This differential relation most likely reflects the fact that subtle cognitive decrements that can be found in non-demented populations do not necessarily evolve into frank cognitive decline in all individuals. Within the population of older persons with microvascular damage, severe cognitive decline probably occurs only in a subgroup of persons, possibly in interaction with other risk factors.[59] Third, concerning brain infarcts, relations between retinal microvascular changes and lacunar infarcts were slightly stronger than with any brain infarcts, which supports the idea that small vessel disease is one of the underlying pathophysiological mechanisms.

In conclusion, there is a consistent relation between the presence of retinal microvascular changes and dementia, cognitive impairment, and brain imaging abnormalities, although more prospective data are needed. Associations are strongest for more severe retinal vascular abnormalities, supporting a role for vascular disease as the underlying pathophysiology of cognitive decline and brain changes. The strength of the relation is modest, particularly for more common retinal microvascular abnormalities. This limits the prognostic value at the population level, however, retinal abnormalities may identify individuals at risk and do offer an important window on the brain for etiological studies.

REFERENCES

1. Viswanathan A, Rocca WA, Tzourio C. Vascular risk factors and dementia: how to move forward? *Neurology*. 2009;72:368–74.
2. Thompson CS, Hakim AM. Living beyond our physiological means: small vessel disease of the brain is an expression of a systemic failure in arteriolar function: a unifying hypothesis. *Stroke*. 2009;40:e322–30.
3. Wong TY, Klein R, Couper DJ, Cooper LS, Shahar E, Hubbard LD, Wofford MR, Sharrett AR. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. *Lancet*. 2001;358:1134–40.
4. Cooper LS, Wong TY, Klein R, Sharrett a R, Bryan RN, Hubbard LD, Couper DJ, Heiss G, Sorlie PD. Retinal microvascular abnormalities and MRI-defined subclinical cerebral infarction: the Atherosclerosis Risk in Communities Study. *Stroke*. 2006;37:82–6.
5. Wong TY, Wong T, Mitchell P. The eye in hypertension. *Lancet*. 2007;369:425–35.
6. Wong TY, McIntosh R. Systemic associations of retinal microvascular signs: a review of recent population-based studies. *Ophthalmic & physiological optics*. 2005;25:195–204.
7. Ding J, Patton N, Deary IJ, Strachan MWJ, Fowkes FGR, Mitchell RJ, Price JF. Retinal microvascular abnormalities and cognitive dysfunction: a systematic review. *The British journal of ophthalmology*. 2008;92:1017–25.
8. Sharrett AR. A review of population-based retinal studies of the microvascular contribution to cerebrovascular diseases. *Ophthalmic epidemiology*. 2007;14:238–42.
9. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ*. 2009;339:b2535–b2535.
10. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of clinical epidemiology*. 2009;62:e1–34.
11. Qiu C, Cotch MF, Sigurdsson S, Jonsson P V, Jonsdottir MK, Sveinbjrnsdottir S, Eiriksdottir G, Klein R, Harris TB, van Buchem M a, Gudnason V, Launer LJ. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. *Neurology*. 2010;75:2221–8.

12. Wong TY, Klein R, Sharrett AR, Nieto FJ, Boland LL, Couper DJ, Mosley TH, Klein BEK, Hubbard LD, Szklo M. Retinal microvascular abnormalities and cognitive impairment in middle-aged persons: the Atherosclerosis Risk in Communities Study. *Stroke*. 2002;33:1487–92.
13. Lesage SR, Mosley TH, Wong TY, Szklo M, Knopman D, Catellier DJ, Cole SR, Klein R, Coresh J, Coker LH, Sharrett a R. Retinal microvascular abnormalities and cognitive decline: the ARIC 14-year follow-up study. *Neurology*. 2009;73:862–8.
14. Liew G, Mitchell P, Wong TY, Lindley RI, Cheung N, Kaushik S, Wang JJ. Retinal microvascular signs and cognitive impairment. *Journal of the American Geriatrics Society*. 2009;57:1892–6.
15. Baker ML, Marino Larsen EK, Kuller LH, Klein R, Klein BEK, Siscovick DS, Bernick C, Manolio TA, Wong TY. Retinal microvascular signs, cognitive function, and dementia in older persons: the Cardiovascular Health Study. *Stroke*. 2007;38:2041–7.
16. Kim DH, Chaves PHM, Newman AB, Klein R, Sarnak MJ, Newton E, Strotmeyer ES, Burke GL, Lipsitz L a. Retinal microvascular signs and disability in the Cardiovascular Health Study. *Archives of ophthalmology*. 2012;130:350–6.
17. Gatto NM, Varma R, Torres M, Wong TY, Johnson PL, Segal-Gidan F, Mack WJ. Retinal microvascular abnormalities and cognitive function in Latino adults in Los Angeles. *Ophthalmic epidemiology*. 2012;19:127–36.
18. Patton N, Pattie A, MacGillivray T, Aslam T, Dhillon B, Gow A, Starr JM, Whalley LJ, Deary IJ. The association between retinal vascular network geometry and cognitive ability in an elderly population. *Investigative ophthalmology & visual science*. 2007;48:1995–2000.
19. De Jong FJ, Schrijvers EMC, Ikram MK, Koudstaal PJ, de Jong PTVM, Hofman a, Vingerling JR, Breteler MMB. Retinal vascular caliber and risk of dementia: the Rotterdam study. *Neurology*. 2011;76:816–21.
20. Schrijvers EMC, Buitendijk GHS, Ikram MK, Koudstaal PJ, Hofman A, Vingerling JR, Breteler MMB. Retinopathy and risk of dementia: the Rotterdam Study. *Neurology*. 2012;79:365–70.
21. Ong SY, Cheung CY, Li X, Lamoureux EL, Ikram MK, Ding J, Cheng CY, Haaland BA, Saw SM, Venketasubramanian N, Chen CPL, Wong TY. Visual impairment, age-related eye diseases, and cognitive function: the Singapore Malay Eye study. *Archives of ophthalmology*. 2012;130:895–900.

22. Cheung CY-L, Ong S, Ikram MK, Ong YT, Chen CP, Venketasubramanian N, Wong TY. Retinal Vascular Fractal Dimension is Associated with Cognitive Dysfunction. *Journal of stroke and cerebrovascular diseases*. 2012;:1–8.
23. Haan M, Espeland MA, Klein BE, Casanova R, Gaussoin SA, Jackson RD, Millen AE, Resnick SM, Rossouw JE, Shumaker SA, Wallace R, Yaffe K. Cognitive function and retinal and ischemic brain changes: the Women's Health Initiative. *Neurology*. 2012;78:942–9.
24. Ding J, Strachan MWJ, Reynolds RM, Frier BM, Deary IJ, Fowkes FGR, Lee AJ, McKnight J, Halpin P, Swa K, Price JF. Diabetic retinopathy and cognitive decline in older people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes*. 2010;59:2883–9.
25. Ding J, Strachan MWJ, Fowkes FGR, Wong TY, Macgillivray TJ, Patton N, Gardiner TA, Deary IJ, Price JF. Association of retinal arteriolar dilatation with lower verbal memory: the Edinburgh Type 2 Diabetes Study. *Diabetologia*. 2011;54:1653–62.
26. De Bresser J, Reijmer YD, van den Berg E, Breedijk MA, Kappelle LJ, Viergever MA, Biessels GJ. Microvascular determinants of cognitive decline and brain volume change in elderly patients with type 2 diabetes. *Dementia and geriatric cognitive disorders*. 2010;30:381–6.
27. Umegaki H, Iimuro S, Kaneko T, Araki A, Sakurai T, Ohashi Y, Iguchi A, Ito H. Factors associated with lower Mini Mental State Examination scores in elderly Japanese diabetes mellitus patients. *Neurobiology of aging*. 2008;29:1022–6.
28. Qiu C, Cotch MF, Sigurdsson S, Klein R, Jonasson F, Klein BEK, Garcia M, Jonsson P V, Harris TB, Eiriksdottir G, Kjartansson O, van Buchem M a, Gudnason V, Launer LJ. Microvascular lesions in the brain and retina: The age, gene/environment susceptibility-Reykjavik study. *Annals of neurology*. 2009;65:569–76.
29. Wong TY, Klein R, Sharrett AR, Couper DJ, Klein BEK, Liao D-P, Hubbard LD, Mosley TH. Cerebral white matter lesions, retinopathy, and incident clinical stroke. *Journal of the American Medical Association*. 2002;288:67–74.
30. Wong TY, Mosley TH, Klein R, Klein BEK, Sharrett AR, Couper DJ, Hubbard LD. Retinal microvascular changes and MRI signs of cerebral atrophy in healthy, middle-aged people. *Neurology*. 2003;61:806–811.

31. Kawasaki R, Cheung N, Mosley T, Islam AFM, Sharrett a R, Klein R, Coker LH, Knopman DS, Shibata DK, Catellier D, Wong TY. Retinal microvascular signs and 10-year risk of cerebral atrophy: the Atherosclerosis Risk in Communities (ARIC) study. *Stroke*. 2010;41:1826–8.
32. Cheung N, Mosley T, Islam A, Kawasaki R, Sharrett AR, Klein R, Coker LH, Knopman DS, Shibata DK, Catellier D, Wong TY. Retinal microvascular abnormalities and subclinical magnetic resonance imaging brain infarct: a prospective study. *Brain*. 2010;133:1987–93.
33. Longstreth W, Larsen EKM, Klein R, Wong TY, Sharrett AR, Lefkowitz D, Manolio TA. Associations between findings on cranial magnetic resonance imaging and retinal photography in the elderly: the Cardiovascular Health Study. *American journal of epidemiology*. 2007;165:78–84.
34. Ikram MK, De Jong FJ, Van Dijk EJ, Prins ND, Hofman A, Breteler MMB, De Jong PTVM. Retinal vessel diameters and cerebral small vessel disease: the Rotterdam Scan Study. *Brain*. 2006;129:182–8.
35. Manschot SM, Biessels GJ, de Valk H, Algra A, Rutten GEHM, van der Grond J, Kappelle LJ. Metabolic and vascular determinants of impaired cognitive performance and abnormalities on brain magnetic resonance imaging in patients with type 2 diabetes. *Diabetologia*. 2007;50:2388–97.
36. Kwon H-M, Kim BJ, Oh JY, Kim SJ, Lee S-H, Oh B-H, Yoon B-W. Retinopathy as an indicator of silent brain infarction in asymptomatic hypertensive subjects. *Journal of the neurological sciences*. 2007;252:159–62.
37. Baker ML, Wang JJ, Liew G, Hand PJ, De Silva DA, Lindley RI, Mitchell P, Wong M-C, Rochtchina E, Wong TY, Wardlaw JM, Hankey GJ. Differential associations of cortical and subcortical cerebral atrophy with retinal vascular signs in patients with acute stroke. *Stroke*. 2010;41:2143–50.
38. Kwa VIH, van der Sande JJ, Stam J, Tijmes N, Vrooland JL. Retinal arterial changes correlate with cerebral small-vessel disease. *Neurology*. 2002;59:1536–1540.
39. Qiu C, Cotch MF, Sigurdsson S, Garcia M, Klein R, Jonasson F, Klein BEK, Eiriksdottir G, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Retinal and cerebral microvascular signs and diabetes: the age, gene/environment susceptibility-Reykjavik study. *Diabetes*. 2008;57:1645–50.
40. Kim DH, Newman AB, Hajjar I, Strotmeyer ES, Klein R, Newton E, Sarnak MJ, Burke GL, Lipsitz LA. Retinal microvascular signs and functional loss in older persons: the cardiovascular health study. *Stroke*. 2011;42:1589–95.

41. Fergenbaum JH, Bruce S, Lou W, Hanley AJG, Greenwood C, Young TK. Window to the brain: Can retinopathy be used to assess cognitive function. *Brain injury*. 2010;24:1448–54.
42. Tekin O, Cukur S, Uraldi C, Işik B, Ozkara A, Kurtaran H, Catal F, Ustün I. Relationship between retinopathy and cognitive impairment among hypertensive subjects. A case-control study in the ankara-pursaklar region. *European neurology*. 2004;52:156–61.
43. Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. *New York: Academic Press*; 1988.
44. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315:629–34.
45. Grading diabetic retinopathy from stereoscopic color fundus photographs--an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. *Ophthalmology*. 1991;98:786–806.
46. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BEK. Revised formulas for summarizing retinal vessel diameters. *Current eye research*. 2003;27:143–9.
47. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV)*. Washington DC: 1994.
48. 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;34:939–44.
49. Chui HC, Victoroff JI, Margolin D, Jagust W, Shankle R, Katzman R. Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. *Neurology*. 1992;42:473–80.
50. Lezak MD, Howieson DB, Bigler ED, Tranel D. *Neuropsychological assessment*. 5th ed. Oxford University Press; 2012.
51. Ikram MK, de Jong FJ, Bos MJ, Vingerling JR, Hofman A, Koudstaal PJ, de Jong PTVM, Breteler MMB. Retinal vessel diameters and risk of stroke: the Rotterdam Study. *Neurology*. 2006;66:1339–43.
52. Wong TY, McIntosh R. Hypertensive retinopathy signs as risk indicators of cardiovascular morbidity and mortality. *British medical bulletin*. 2005;73-74:57–70.

53. Mimoun L, Massin P, Steg G. Retinal microvascularisation abnormalities and cardiovascular risk. *Archives of cardiovascular diseases*. 2009;102:449–56.
54. Henderson AD, Bruce BB, Newman NJ, Biousse V. Hypertension-related eye abnormalities and the risk of stroke. *Reviews in neurological diseases*. 2011;8:1–9.
55. Grosso A, Cheung N, Veglio F, Wong TY. Similarities and differences in early retinal phenotypes in hypertension and diabetes. *Journal of hypertension*. 2011;29:1667–75.
56. Cheung CY-L, Ikram MK, Sabanayagam C, Wong TY. Retinal microvasculature as a model to study the manifestations of hypertension. *Hypertension*. 2012;60:1094–103.
57. Wong TY. Retinal Vessel Diameters and Their Associations with Age and Blood Pressure. *Investigative Ophthalmology & Visual Science*. 2003;44:4644–4650.
58. Kloppenborg RP, van den Berg E, Kappelle LJ, Biessels GJ. Diabetes and other vascular risk factors for dementia: which factor matters most? A systematic review. *European journal of pharmacology*. 2008;585:97–108.
59. Van den Berg E, Reijmer YD, de Bresser J, Kessels RPC, Kappelle LJ, Biessels GJ. A 4 year follow-up study of cognitive functioning in patients with type 2 diabetes mellitus. *Diabetologia*. 2010;53:58–65.

APPENDIX 1

Classification of cognitive domains and included tests

Memory

Immediate and Delayed Word Recall (CLVT, California Verbal Learning Test)
Logical Memory (WMS-III, Wechsler Memory Scale-III)
Faces and family subtest (WMS-III)
Delayed Word Recall Test

Processing speed

Digit Symbol Substitution Test (WAIS, Wechsler Adult Intelligence Scale)
Salthouse Figure Comparison Test
Stroop Color Word Test part I and II

Executive functioning and attention

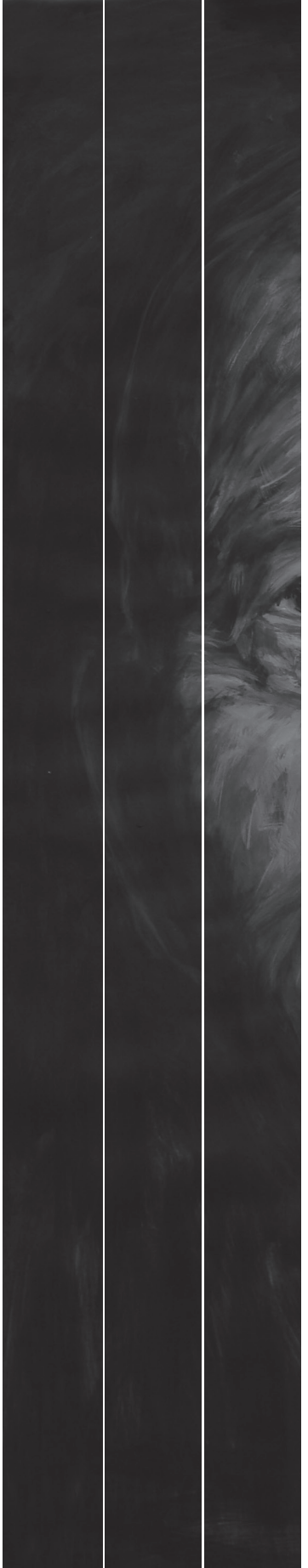
Stroop Color Word Test part III
Word Fluency Test
COWAT Phonemic Fluency
Trail Making Test (TMT) part B
Digit Span Backward
Shortened version of the Spatial Working Memory Test (CANTAB, Cambridge Neuropsychological Test Automated Battery)

Fluid intelligence

Raven's Standard Progressive Matrices
Matrix reasoning (WAIS)

Mental state / dementia screening

Mini-Mental State Examination (MMSE)
Modified MMSE (3MSE)
Abbreviated Mental Test (AMT)
Cognitive Abilities Screening Instrument, abbreviated version (CASI-S)



CHAPTER 3

ALBUMINURIA AND COGNITIVE FUNCTIONING IN AN OLDER POPULATION: THE HOORN STUDY

SM Heringa, E van den Berg, JM Dekker, G Nijpels, RPC Kessels,
LJ Kappelle, CDA Stehouwer, GJ Biessels

Published in: Dementia and Geriatric Cognitive Disorders
2011;32(3):182-7.

ABSTRACT

BACKGROUND: Markers of vascular disease elsewhere in the body may reflect vascular abnormalities in the brain relevant to age-related cognitive decline and dementia. We examined the association between albuminuria, as a marker of microvascular damage, and cognition in older individuals.

METHODS: 380 individuals (age 73±6 years), participating in the population-based Hoorn study, underwent extensive neuropsychological examination in 2005-2008, and urinary albumin-to-creatinine ratios measurements in 2000-2001 (n=378) and/or 2005-2008 (n=346). Cognition was expressed in z-scores on six domains.

RESULTS: In 2000-2001 42 participants were with and 336 without albuminuria, in 2005-2008 51 with and 295 without. In age-, sex- and premorbid IQ-adjusted analyses participants with albuminuria 5-7 years earlier had slightly lower z-scores for the domains attention and executive functioning (mean difference: -0.21 (95% CI: -0.40 to -0.02)) and language (-0.36 (-0.63 to -0.09)). No statistically significant differences in cognition were found between participants with and without albuminuria at the time of neuropsychological testing.

CONCLUSION: Albuminuria predicts future modest cognitive decrements, but concurrent albuminuria is unrelated to cognitive functioning. The link between albuminuria and cognitive dysfunction may convey an etiological message, but because effect sizes were modest its value in prognostic models for cognitive decline may be limited.

INTRODUCTION

The role of vascular disease in cognitive decline and dementia in older people is increasingly recognized. Vascular risk factors, such as hypertension, and vascular damage in the brain clearly contribute to dementia risk.[1] Because vascular disease elsewhere in the body is associated with vascular abnormalities in the brain,[2] measures of vascular damage in other organs may serve as biomarkers of vascular-mediated cognitive dysfunction. Albuminuria is a marker of renal microvascular disease and of endothelial dysfunction and is clearly related to exposure to vascular risk factors such as hypertension.[3] Thus far, studies on the association between albuminuria and cognition in the general population have shown variable results.[4–8] In the current study we examined the relationship between cognitive functioning and albuminuria, at the time of a detailed neuropsychological assessment and 5-7 years prior to this assessment, in a longitudinal cohort of older persons.

METHODS

Participants

The Hoorn study is a population-based cohort study on glucose metabolism, which started in 1989 and included 2,484 participants aged 50 to 75 years at baseline. Follow-up examinations of this cohort were performed in 1996-1998, 2000-2001 and 2005-2008. Details on the design of the baseline study[9] and the follow-up examinations[10–12] have been described elsewhere. Albuminuria was determined in 2000-2001 (n=644) and in 2005-2008 (n=350), cognitive functioning was assessed in 2005-2008 (n=385).

For the present analysis we included all persons with assessment of cognitive functioning and at least one urinary albumin-to-creatinine ratio measurement. Five participants were excluded because of unreliable neuropsychological examination (e.g. hearing or language difficulties). Of the remaining 380 participants, albumin-to-creatinine ratios in the urine could be measured in 378 persons in 2000-2001, and in 346 persons in 2005-2008.

The Hoorn study was approved by the medical ethics committee of the VU University Medical Center and was performed in accordance with the

guidelines of the Helsinki Declaration. Written informed consent was obtained from all participants.

Measurements

Both at the 2000-2001 and 2005-2008 examinations participants underwent a standardized interview, physical examination and blood tests. Weight (kg) and height (cm) were measured in participants wearing light clothes. Body mass index (BMI) was calculated as weight divided by squared height. Blood pressure (mmHg) was measured in the right arm with a random-zero sphygmomanometer while participants were sitting. Systolic blood pressure and diastolic blood pressure were calculated as the mean of duplicate measurements. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or use of blood pressure lowering medication. Glycated hemoglobin level (HbA1c, %) was determined by ion-exchange high-performance liquid. The presence of type 2 diabetes mellitus was based on WHO-criteria.13 Total cholesterol was determined by enzymatic techniques (Roche, Mannheim, Germany). All blood samples were analyzed at the clinical chemistry laboratory of the VU University Medical Center. Self-reported information on the participants' current use of medications, medical history and smoking status (current Y/N) was obtained by a standardized questionnaire. History of cardiovascular disease was defined as self-reported intermittent claudication, angina pectoris, possible myocardial infarction, amputation, stroke or transient ischemic attack.

Albumin-to-creatinine ratios were calculated to determine the presence of albuminuria. Urinary albumin was measured by rate nephelometry (Array Protein System; Beckman, Galway, Ireland). Urinary creatinine was measured by means of a modified Jaffé method. Participants were classified as having albuminuria if they had an albumin-to-creatinine ratio ≥2.0 mg/mmol.[14,15]

Neuropsychological assessment

At the 2005-2008 examination, all participants performed an extensive neuropsychological examination including twelve verbal and non-verbal tasks, administered in a fixed order that took 90 minutes to complete.[16] The tasks were divided into six cognitive domains to reduce the amount of neuropsychological variables and for clinical clarity. This division was made a priori, according to

standard neuropsychological practice and cognitive theory, as described in detail in Lezak.[17] The domain abstract reasoning was assessed by Raven Advanced Progressive Matrices (12-item short form). The domain memory included four subdomains: working memory assessed by the forward and backward digit span of the Wechsler Adult Intelligence Scale-III (WAIS-III) and the Corsi Block-Tapping Task; immediate memory and learning rate, including verbal memory assessed by the Rey Auditory Verbal Learning Test and visual memory assessed by the Location Learning Test; forgetting rate assessed by the delayed task of the Rey Auditory Verbal Learning Test and of the Location Learning Test; and incidental memory assessed by the delayed trial of the Rey-Osterrieth Complex Figure. The domain information processing speed was assessed by the Trail Making Test Part A, the Stroop Color-Word Test (Parts I and II), and the subtest Digit Symbol of the WAIS-III. The domain attention and executive function was assessed by the Trail Making Test Part B, the Stroop Color-Word Test (Part III), the Brixton Spatial Anticipation Test, a verbal fluency test using the N and A, and category fluency using animal names. The domain visuoconstruction was assessed by the copy trial of the Rey-Osterrieth Complex Figure. The domain language was assessed by the Token Test (short form). To compare the six different cognitive domains between the groups the raw test scores were standardized into z-scores per test. These z-scores were calculated on the pooled mean of the test performance of the whole study population. The z-score for each domain was derived by calculating the mean of the z-scores of the tests comprising that domain. A sum score was also calculated, representing the mean z-score over the six cognitive domains as a measure of overall cognitive functioning. The Dutch version of the National Adult Reading Test was used as an estimation of the premorbid level of intellectual functioning. Educational level was recorded (7 categories). Depressive symptoms were assessed with the validated Dutch version of the 20-item Center for Epidemiologic Studies Depression Scale (CES-D; possible range 0 to 60). A score ≥16 was considered an indicator of possible depression.

Statistical analysis

Between-group differences in population characteristics were analyzed with analysis of variance for continuous variables, Mann-Whitney U tests for non-parametric data and chi-square tests for proportions. Between-group differences

in cognitive functioning (z-scores) on the six cognitive domains and the sum score were examined with analysis of covariance, with sex, age and estimated premorbid IQ included as covariates. The prospective analysis was performed by comparing cognitive functioning between participants with and without albuminuria in 2000-2001. For the cross-sectional analysis cognitive functioning was compared between participants with and without albuminuria in 2005-2008. We examined whether the predictive value (longitudinal analysis) or relation (cross-sectional analysis) between albuminuria and cognition was influenced by factors known to be associated with albuminuria and cognition. These factors were blood pressure and the use of antihypertensive medication, type 2 diabetes (patients with type 2 diabetes were oversampled during the 2000-2001 measurement[18]), and the presence of cardiovascular disease or depression.

Secondary analyses were performed to explore dose-response relations between log transformed albumin-to-creatinine ratio (ACR) in 2000-2001 and 2005-2008 and cognitive functioning.

Because only few participants developed albuminuria between the examinations of 2000-2001 and 2005-2008, analysis on the relation between incident albuminuria and cognition was not performed.

RESULTS

Urine samples were available from 644 participants of the 2000-2001 examination. The proportion of persons with albuminuria was higher in those not participating in the 2005-2008 examination (53/266=20%) than in those who did participate (42/378=11%; chi^2 (1) = 9.645, p=0.001). All-cause mortality was also higher in persons with albuminuria in 2000-2001 compared to those without (29 (31%) versus 80 (15%); age- and sex-adjusted odds ratio 1.65 (95% confidence interval (CI) 0.97-2.83, p=0.066)).

Table 1 shows the characteristics of the participants with and without albuminuria in 2000-2001 and 2005-2008, who underwent cognitive assessment at the 2005-2008 examination. None of the participants had cognitive dysfunction severe enough to disturb day-to-day functioning. Z-scores for the cognitive domains in the participants with and without albuminuria were normally distributed. Of the 378 participants with urine

Table 1. Characteristics of participants

	2000-2001 n=378		2005-2008 n=346	
	With albuminuria	Without albuminuria	With albuminuria	Without albuminuria
N	42	336	51	295
Male, %	24 (57%)	169 (50%)	36 (71%)	144 (49%) *
Age, y	70.1 ± 6.8	67.4 ± 5.2 *	74.1 ± 6.6	72.4 ± 5.5 *
Education, median (IQR)[a,b]	4 (4-5)	4 (4-5)	4 (4-5)	4 (4-5)
Estimated premorbid IQ, points[a]	98 ± 13	97 ± 15	95 ± 14	99 ± 13 *
Type 2 Diabetes Mellitus	13 (31%)	59 (18%)	23 (47%)	53 (19%) **
HbA1c, %	6.2 ± 0.0	5.9 ± 0.6 *	6.1 ± 0.8	5.7 ± 0.5 **
Total cholesterol, mmol/l	5.6 ± 1.0	5.7 ± 1.0	5.2 ± 1.1	5.3± 1.1
Systolic blood pressure (mmHg)	145 ± 18.9	139 ± 19.1	154 ± 20	144 ± 20 *
Diastolic blood pressure (mmHg)	85 ± 9.9	82 ± 11.3	78 ± 12	74 ± 11 *
Use of antihypertensive medication, %	21 (50%)	102 (30%) *	25 (49%)	112 (38%)
Hypertension[c]	34 (81%)	206 (61%) *	43 (84%)	202 (69%) *
History of cardiovascular disease[d]	7 (17%)	45 (13%)	11 (22%)	51 (17%)
Current smokers	5 (12%)	38 (11%)	6 (12%)	36 (12%)
Depressive symptoms (CES-D ≥ 16)[a]	-	-	5 (10%)	28 (10%)

* significant at the 0.05 level between participants with and without albuminuria

** significant at the 0.001 level between participants with and without albuminuria

Data are presented as mean ± SD, or n (%) unless otherwise specified.

IQR, interquartile range; IQ, intelligence quotient

[a] measured in 2005-2008

[b] seven categories

[c] defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or use of blood pressure lowering medication

[d] defined as self- reported history of claudicatio intermittens, angina pectoris, possible myocardial infarction, amputation (Rose questionnaire[19]), or stroke/transient ischemic attack

samples at the 2000-2001 examination 42 (11%) had albuminuria. Individuals with albuminuria were older, had higher HbA1c levels and more frequently used anti-hypertensive medication (Table 1).

Persons with albuminuria at the 2000-2001 examination had significantly lower z-scores for the cognitive domains attention and executive functioning (mean difference adjusted for age, sex and estimated premorbid IQ -0.21 (95% CI -0.40 to -0.02), p=0.027,) and language (-0.36 (95% CI -0.63 to -0.09), p=0.009) and a trend towards lower information processing speed (-0.21 (95% CI -0.42 to 0.02), p=0.075). The other domains did not differ significantly (Table 2). Additional adjustment for depressive symptoms, hypertension, systolic blood pressure, use of anti-hypertensive medication, cardiovascular disease or type 2 diabetes and exclusion of subjects with an albumin-to-creatinine ratio > 25 mg/mmol (i.e., macroalbuminuria) did not notably change the results (data not shown). Secondary analyses, between 2000-2001 ACR as a continuous variable and cognition in 2005-2008 yielded no statistically significant associations (Table 3).

Of the 346 participants with urine samples and cognitive assessment at the 2005-2008 examination 51 (15%) had albuminuria. Participants with albuminuria were more frequently male, were older, had a lower estimated premorbid IQ, higher HbA1c levels, had higher blood pressures and were more likely to have type 2 diabetes (Table 1). The cognitive domain scores did not differ between participants with and without albuminuria (Table 2). Mean differences in z-scores were in the -0.2 to 0.2 range. In this cross-sectional analysis, like in the prospective analysis, the results were not notably changed by additional adjustment for depressive symptoms, hypertension, systolic blood pressure, use of anti-hypertensive medication, cardiovascular disease or type 2 diabetes or after excluding participants with macroalbuminuria (data not shown). Secondary analyses between 2005-2008 ACR as a continuous variable and concurrent cognition yielded no statistically significant associations (Table 3).

DISCUSSION

The present study shows that in a population-based sample of older persons participating in the Hoorn study albuminuria 5-7 years prior to the cognitive assessment was associated with modest cognitive decrements on the domains

Table 2. Comparison of cognitive functioning in 2005-2008 between participants with and without albuminuria in 2000-2001 and in 2005-2008 (no albuminuria = reference)

	2000-2001 (n=378)	**2005-2008 (n=346)**
	Mean difference in z-scores[1]	Mean difference in z-scores[1]
	Beta (95% confidence interval)	Beta (95% confidence interval)
Abstract reasoning	0.24 (-0.08 to 0.56)	0.02 (-0.27 to 0.30)
Memory	-0.06 (-0.20 to 0.08)	-0.02 (-0.16 to 0.12)
Information processing speed	-0.21 (-0.45 to 0.02)	-0.02 (-0.24 to 0.21)
Attention & Executive functioning	-0.21 (-0.40 to -0.02) *	-0.13 (-0.30 to 0.04)
Visuoconstruction	0.20 (-0.12 to 0.51)	0.11 (-0.19 to 0.40)
Language	-0.36 (-0.63 to -0.09) *	-0.14 (-0.40 to 0.12)
Sum score	-0.09 (-0.23 to 0.05)	-0.04 (-0.17 to 0.10)

[1] adjusted for sex, age and estimated premorbid IQ

* significant at the 0.05 level

Table 3. Relation between log transformed Albumin-to-Creatinine Ratios in 2000-2001 and in 2005-2008 and Cognitive Functioning in 2005-2008

	2000-2001 (n=378)	**2005-2008 (n=346)**
	Beta (95% confidence interval)	Beta (95% confidence interval)
Abstract reasoning	0.07 (-0.03 to 0.16)	0.02 (-0.08 to 0.12)
Memory	-0.04 (-0.13 to 0.05)	0.00 (-0.10 to 0.10)
Information processing speed	-0.10 (-0.18 to 0.02)	-0.07 (-0.16 to 0.01)
Attention & Executive functioning	-0.10 (-0.19 to 0.01)	-0.09 (-0.18 to 0.01)
Visuoconstruction	0.05 (-0.04 to 0.15)	0.04 (-0.06 to 0.14)
Language	-0.06 (-0.15 to 0.03)	-0.09 (-0.18 to 0.00)
Sum score	-0.04 (-0.11 to 0.04)	-0.05 (-0.13 to 0.03)

[1] adjusted for sex, age and estimated premorbid IQ

attention and executive function, and language. However, albuminuria measured at the time of the cognitive assessment was unrelated to cognition.

Several cross-sectional studies have found a relation between albuminuria and cognitive functioning in the general population. One study reported a higher prevalence of dementia (OR 1.20; 95% CI 1.15 to 1.29) in persons with albuminuria compared to those without.[4] Others reported worse executive functioning[5] and a lower information processing speed (NHANES study[20]) in individuals with albuminuria as compared to those without. The relation with cognition has also been studied prospectively. A recent large study (n=28,384)[8] demonstrated an increased risk of Mini-Metal State Examination (MMSE) scores below 24 for participants with baseline microalbuminuria and macroalbuminuria, as well as an increased risk of cognitive decline of more than 3 MMSE points after five years. A study in 759 older persons[7] did not find an association between albumin-to-creatinine ratio and cognition at baseline, but after 6.6 years follow up baseline albuminuria was associated with accelerated decline in MMSE scores and executive functioning, only in men. In another longitudinal survey studying older persons with impaired glucose tolerance albuminuria was associated with an increased risk of cognitive decline (defined as a composite score of attention and executive functions) after 12 months.[21] In this latter study, arterial stiffness influenced the association between microalbuminuria and cognition. In addition, the NHANES showed that the relation between microalbuminuria and cognition was particularly evident in persons with peripheral arterial disease,[22] a result that could not be confirmed in the present study. All in all, albuminuria can be considered a predictor of cognitive decline in the years to come. It is important to note, however, that effect sizes are small. In our study mean performance in 2005-2008 of patients with albuminuria in 2000-2001 was at the 35-45th percentile of controls (effect size 0.2 to 0.3). Also, on the domains that were not significantly affected, the 95% CIs of the effect sizes for the difference in performance between people with and without albuminuria were narrow. This was also supported by the secondary analyses, with ACR as a continuous variable, were the values of the standardized betas were low. This indicates that the association between albuminuria and cognition in this population is only modest: in neuropsychological studies, effect sizes below 0.2 are considered small, between 0.2 and 0.8 medium and above 0.8

large.[23] Nevertheless, it is interesting to note that the cognitive functions that appear to be most affected are more related to executive functioning / subcortical functions rather than, for example, memory functioning. This distinction has been reported before in populations with vascular damage.[24] We also found that people with albuminuria were more likely to die. This may limit the relevance and usefulness of microalbuminuria as a predictor of cognitive decline in prognostic models. Nevertheless, our findings do convey an etiological message: the microvasculature of the kidney and the brain are exposed to the same risk factors, such as hypertension, diabetes or hypercholesterolemia.[2] Hence, although albuminuria does not reflect a single etiological process, microvascular damage in the kidney and the brain may develop side by side and reflect exposure to vascular risk and susceptibility to microvascular damage.

Strengths of the present study include the detailed recording of vascular and metabolic determinants and cognitive functioning over an extended period in a well-defined population-based cohort of older participants. The detailed neuropsychological assessment was administered to a large number of individuals, which allowed us to detect subtle differences in performance between the groups with and without albuminuria (i.e. between group differences in z-score of 0.21 to 0.36). Hence, the absence of an association between albuminuria and cognition in the cross-sectional analysis was not due to low statistical power. Limitations include the absence of creatinine measurements in serum, as a result of which eGFR as a measure of kidney function could not be determined. Consequently, previous findings showing a nonlinear cross-sectional association between eGFR and prevalence of cognitive impairment[25] could not be replicated. A possible selection bias may have occurred due to attrition of this elderly population during follow-up, since persons with a less favorable vascular risk factor profile at baseline (1989) or severe cognitive dysfunction are more likely to be lost to follow-up.

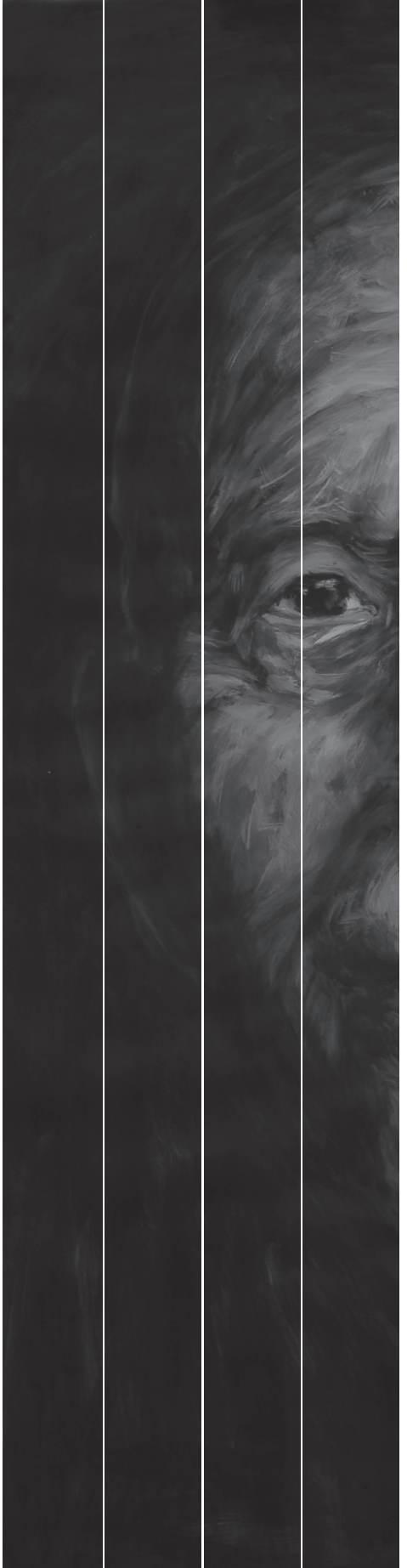
In conclusion, albuminuria is associated with modest cognitive dysfunction 6 years later. Concurrent albuminuria is unrelated to cognitive functioning in a population-based cohort of older individuals. The link between albuminuria and cognitive dysfunction may convey an etiological message, but the relevance of albuminuria in prognostic models for cognitive decline may be limited, because albuminuria is also a marker of increased mortality, and because effect sizes in survivors are small.

REFERENCES

1. Hachinski V. Shifts in thinking about dementia. *Journal of the American Medical Association*. 2008;300:2172–3.
2. Knopman DS. Invited commentary: Albuminuria and microvascular disease of the brain--a shared pathophysiology. *American journal of epidemiology*. 2010;171:287–9; author reply 290–1.
3. Stehouwer C, Smulders YM. Microalbuminuria and risk for cardiovascular disease: Analysis of potential mechanisms. *Journal of the American Society of Nephrology*. 2006;17:2106–11.
4. Barzilay JI, Fitzpatrick AL, Luchsinger J, Yasar S, Bernick C, Jenny NS, Kuller LH. Albuminuria and dementia in the elderly: a community study. *American journal of kidney diseases*. 2008;52:216–26.
5. Weiner DE, Bartolomei K, Scott T, Price LL, Griffith JL, Rosenberg I, Levey AS, Folstein MF, Sarnak MJ. Albuminuria, cognitive functioning, and white matter hyperintensities in homebound elders. *American journal of kidney diseases*. 2009;53:438–47.
6. Triantafyllidi H, Arvaniti C, Lekakis J, Ikonomidis I, Siafakas N, Tzortzis S, Trivilou P, Zerva L, Stamboulis E, Kremastinos DT. Cognitive impairment is related to increased arterial stiffness and microvascular damage in patients with never-treated essential hypertension. *American journal of hypertension*. 2009;22:525–30.
7. Jassal SK, Kritz-Silverstein D, Barrett-Connor E. A prospective study of albuminuria and cognitive function in older adults: the Rancho Bernardo study. *American journal of epidemiology*. 2010;171:277–86.
8. Barzilay JI, Gao P, O'Donnell M, Mann JFE, Anderson C, Fagard R, Probstfield J, Dagenais GR, Teo K, Yusuf S. Albuminuria and decline in cognitive function: The ONTARGET/TRANSCEND studies. *Archives of internal medicine*. 2011;171:142–50.
9. Mooy JM, Grootenhuis PA, de Vries H, Valkenburg HA, Bouter LM, Kostense PJ, Heine RJ. Prevalence and determinants of glucose intolerance in a Dutch caucasian population. The Hoorn Study. *Diabetes care*. 1995;18:1270–3.
10. Henry RMA, Kostense PJ, Spijkerman AMW, Dekker JM, Nijpels G, Heine RJ, Kamp O, Westerhof N, Bouter LM, Stehouwer CDA. Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study. *Circulation*. 2003;107:2089–95.

11. De Vegt F, Dekker JM, Jager A, Hienkens E, Kostense PJ, Stehouwer CDA, Nijpels G, Bouter LM, Heine RJ. Relation of impaired fasting and postload glucose with incident type 2 diabetes in a Dutch population: The Hoorn Study. *Journal of the American Medical Association*. 2001;285:2109–13.
12. Van den Berg E, Dekker JM, Nijpels G, Kessels RPC, Kappelle LJ, de Haan EHF, Heine RJ, Stehouwer CDA, Biessels GJ. Cognitive functioning in elderly persons with type 2 diabetes and metabolic syndrome: the Hoorn study. *Dementia and geriatric cognitive disorders*. 2008;26:261–9.
13. World Health Organization. *Definition, diagnosis and classification of diabetes mellitus: report of a WHO Consultation* (WHO / NCD / NCS/99.2. 1999). 1999.
14. Jager A, Kostense PJ, Nijpels G, Heine RJ, Bouter LM, Stehouwer CDA. Microalbuminuria is strongly associated with NIDDM and hypertension, but not with the insulin resistance syndrome: the Hoorn Study. *Diabetologia*. 1998;41:694–700.
15. Gatling W, Knight C, Mullee MA, Hill RD. Microalbuminuria in diabetes: a population study of the prevalence and an assessment of three screening tests. *Diabetic medicine*. 1988;5:343–7.
16. Brands AMA, Van den Berg E, Manschot SM, Biessels GJ, Kappelle LJ, De Haan EHF, Kessels RPC. A detailed profile of cognitive dysfunction and its relation to psychological distress in patients with type 2 diabetes mellitus. *Journal of the International Neuropsychological Society*. 2007;13:288–97.
17. Lezak MD, Howieson DB, Loring DW. *Neuropsychological Assessment*. 4th ed. Oxford University Press, 2004.
18. Henry RMA, Ferreira I, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Kamp O, Bouter LM, Stehouwer CDA. Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not; The Hoorn Study. *Atherosclerosis*. 2004;174:49–56.
19. Rose G, Blackburn H, Gillum R. *Cardiovascular Survey Methods*. Geneva, WHO Press. 1982.
20. Kuo H-K, Lin L-Y, Yu Y-H. Microalbuminuria is a negative correlate for cognitive function in older adults with peripheral arterial disease: results from the U.S. National Health and Nutrition Examination Survey 1999-2002. *Journal of internal medicine*. 2007;262:562–70.

21. Abbatecola AM, Barbieri M, Rizzo MR, Grella R, Laieta MT, Quaranta E, Molinari AM, Cioffi M, Fioretto P, Paolisso G. Arterial stiffness and cognition in elderly persons with impaired glucose tolerance and microalbuminuria. *The journals of gerontology. Series A, Biological sciences and medical sciences*. 2008;63:991–6.
22. Vupputuri S, Shoham DA, Hogan SL, Kshirsagar A. Microalbuminuria, peripheral artery disease, and cognitive function. *Kidney international*. 2008;73:341–6.
23. Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd ed. New York: Academic Press; 1988.
24. Prins ND, van Dijk EJ, den Heijer T, Vermeer SE, Jolles J, Koudstaal PJ, Hofman A, Breteler MMB. Cerebral small-vessel disease and decline in information processing speed, executive function and memory. *Brain*. 2005;128:2034–41.
25. Kurella Tamura M, Wadley V, Yaffe K, McClure LA, Howard G, Go R, Allman RM, Warnock DG, McClellan W. Kidney function and cognitive impairment in US adults: the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study. *American journal of kidney diseases*. 2008;52:227–34.



CHAPTER 4

MARKERS OF LOW-GRADE INFLAMMATION AND ENDOTHELIAL DYSFUNCTION ARE RELATED TO REDUCED INFORMATION PROCESSING SPEED AND EXECUTIVE FUNCTIONING IN AN OLDER POPULATION – THE HOORN STUDY

SM Heringa, E van den Berg, YD Reijmer, G Nijpels, CDA Stehouwer, CG Schalkwijk, T Teerlink, PG Scheffer, K van den Hurk, LJ Kappelle, JM Dekker, GJ Biessels

Published in: Psychoneuroendocrinology 2014;40:108-118.

ABSTRACT

BACKGROUND: Low-grade inflammation and endothelial dysfunction are related to cognitive decline and dementia, in a complex interplay with vascular factors and aging. We investigated, in an older population, low-grade inflammation and endothelial dysfunction in relation to detailed assessment of cognitive functioning. Furthermore, we explored this association within the context of vascular factors.

METHODS: 377 participants (73±6 years) of the population-based Hoorn Study were included. In plasma samples of 2000-2001 (n=363) and/or 2005-2008 (n=323), biomarkers were determined of low-grade inflammation (CRP, TNF-α, IL-6, IL-8, SAA, MPO, and sICAM-1) and endothelial dysfunction (vWF, sICAM-1, sVCAM-1, sTM, sE-selectin). In 2005-2008, all participants underwent neuropsychological examination. Composite z-scores were computed for low-grade inflammation and endothelial dysfunction at both time points, and for six domains of cognitive functioning (abstract reasoning, memory, information processing speed, attention and executive functioning, visuoconstruction, and language). The association between low-grade inflammation and endothelial dysfunction, and cognitive functioning was evaluated with linear regression analysis. In secondary analyses, we explored the relation with vascular risk factors and cardiovascular disease.

RESULTS: Low-grade inflammation and endothelial dysfunction were associated with worse performance on information processing speed and attention and executive functioning, in prospective and cross-sectional analyses (standardized betas ranging from -0.20 to -0.10). No significant relation with other cognitive domains was observed. Adjusting for vascular factors slightly attenuated the associations. Low-grade inflammation and endothelial dysfunction accounted for only 2.6% explained variance in cognitive functioning, on top of related vascular risk factors and cardiovascular disease. Bootstrapping analyses show that low-grade inflammation and endothelial dysfunction mediate the relation between vascular risk factors and cognitive functioning.

CONCLUSION: This study shows that low-grade inflammation and endothelial dysfunction contribute to reduced information processing speed and executive functioning in an older population.

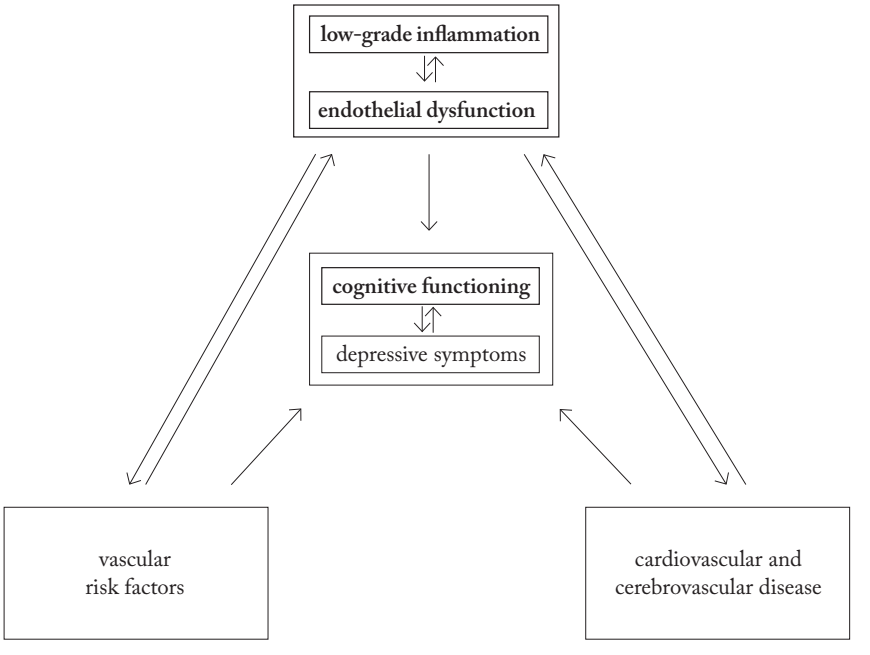
INTRODUCTION

Cognitive impairment and dementia are important public health problems. To date, no cure is available, but some factors contributing to cognitive decline are potentially modifiable. These include vascular risk factors such as diabetes, hypertension, dyslipidemia, obesity, and smoking, which are part of a complex cascade of subclinical vascular abnormalities that occurs in aging.[1] Eventually, such abnormalities can lead to cardiovascular events such as ischemic heart disease and stroke, but also to more insidious global changes in the brain and to cognitive decrements. In this vascular cascade, chronic low-grade inflammation and endothelial dysfunction are thought to play an important role.[2]

The relation between low-grade inflammation and endothelial dysfunction, vascular risk factors, cardiovascular disease, and cognitive functioning is complex (see figure). Low-grade inflammation and endothelial dysfunction are closely linked: inflammatory cytokines can induce endothelial dysfunction, and endothelial dysfunction is a pro-inflammatory state.[3] These processes may have a direct effect on the brain due to their role in neurotransmitter and neuroendocrine responses.[4] However, low-grade inflammation and endothelial dysfunction may also affect the brain through vascular insufficiency. Low-grade inflammation, endothelial dysfunction and vascular risk factors reinforce each other[5–7] and can lead to cardiovascular and cerebrovascular disease, by themselves and in interaction.[7,8] Furthermore, ischemia as a result of vascular disease may lead to an inflammatory response.[9] Moreover, inflammatory and vascular disease do not only result in cognitive decrements but also in depressive symptoms,[10] which are known to interact with cognitive performance.[11,12]

Low-grade inflammation and endothelial dysfunction could thus have their own influence on cognitive performance and at the same time may be intermediate processes between vascular risk factors, cardiovascular disease and cognitive impairment. Results from previous studies provide evidence for associations between biomarkers of inflammation and/or endothelial dysfunction and cognitive dysfunctioning.[2,13–16] To our knowledge however, thus far no studies have addressed this relation by combining multiple circulating biomarkers for both low-grade inflammation and endothelial dysfunction with extensive neuropsychological examination on a wide range of cognitive domains.

Figure. Relation between low-grade inflammation and endothelial dysfunction, vascular factors, cognitive functioning, and depressive symptoms



Low-grade inflammation and endothelial dysfunction can contribute to changes in cognitive function and mood, both through direct effects on the brain, and in interaction with vascular risk factors and cardiovascular and cerebrovascular disease (for details and references see introduction). This complex interplay forms the framework for the analyses in this paper, where we examine 1) the relation between low-grade inflammation/endothelial dysfunction and cognitive functioning; 2) the independence of this relation from vascular risk factors, cardiovascular disease, or depressive symptoms; 3) the mediating effect of low-grade inflammation/endothelial dysfunction on the relation between vascular risk factors and cognitive functioning.

The aim of the present study was to examine the relation between circulating biomarkers of low-grade inflammation and of endothelial dysfunction and a detailed assessment of cognitive functioning, in a population-based sample of older individuals. We provide additional analyses to further explore the complex relation between low-grade inflammation, endothelial dysfunction, cognitive functioning, vascular factors, and depressive symptoms.

METHODS

Participants

The Hoorn Study is a population-based cohort study on glucose metabolism, which started in 1989 and included 2,484 participants aged 50-75 years at baseline. Follow-up examinations of this cohort were performed in 1996-1998, 2000-2001 and 2005-2008. Details on the design of the baseline study and the follow-up have been described elsewhere.[17–19] Circulating biomarkers of low-grade inflammation and endothelial dysfunction were determined in 2000-2001 (n=765) and again in 2005-2008 (n=450), cognitive functioning was assessed in 2005-2008 (n=385). For the present analyses, we included all persons with at least one measurement of circulating biomarkers and with assessment of cognitive functioning. Five participants were excluded because of unreliable neuropsychological examination (e.g. hearing or language difficulties). Of the remaining 377 participants, biomarkers of low-grade inflammation and endothelial dysfunction were measured in 363 persons in 2000-2001 and in 323 persons in 2005-2008. The Hoorn Study was approved by the medical ethics committee of the VU University Medical Center and was performed in accordance with the guidelines of the Helsinki Declaration. Written informed consent was obtained from all participants.

Measurements

Both at the 2000-2001 and 2005-2008 examinations, participants underwent a standardized interview, physical examination, and blood tests. Weight (kg) and height (cm) were measured in participants wearing light clothes. Body mass index was calculated as weight divided by squared height. Blood pressure (mmHg)

was measured in the right arm with a random-zero sphygmomanometer while participants were sitting. Systolic blood pressure and diastolic blood pressure were calculated as the mean of duplicate measurements. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or use of blood pressure-lowering medication. Glycated hemoglobin level (HbA1c, %) was determined by ion-exchange high-performance liquid chromatography. The presence of type 2 diabetes mellitus was based on WHO criteria (1999). Total cholesterol was determined by enzymatic techniques (Roche, Mannheim, Germany). All blood samples were analyzed at the clinical chemistry laboratory of the VU University Medical Center. Self-reported information on the participants' current use of medications, medical history, and smoking status (current yes/no) was obtained by a standardized questionnaire. History of cardiovascular disease was defined as self-reported intermittent claudication, angina pectoris, possible myocardial infarction, amputation, stroke, or transient ischemic attack (Rose questionnaire[20]).

Low-grade inflammation and endothelial dysfunction

Low-grade inflammation is reflected in modest elevations of acute-phase reactants such as C-reactive protein (CRP) and serum amyloid A (SAA), cytokines such as tumor necrosis factor alpha (TNF-α), interkleukin-6 (IL-6), and interleukin-8 (IL-8), the enzyme myeloperoxidase (MPO), and the adhesion molecule soluble intercellular adhesion molecule-1 (sICAM-1).[21,22] The endothelium has many functions and is heterogeneous, the concept of endothelial dysfunction therefore has many dimensions.[23,24] Circulating markers, such as von Willebrand factor (vWf), soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble endothelial selectin (sE-selectin), soluble thrombomodulin (sTM), and sICAM-1 are synthesized by endothelial cells and higher circulating concentrations reflect dysfunction of the endothelium.[21,22] Combinations of these markers are considered to give valid overall estimates of respectively low-grade inflammation or endothelial dysfunction. We therefore included high-sensitivity CRP, SAA, IL-6, IL-8, TNF-α, sICAM-1, and, in 2000-2001 only, MPO as markers of low-grade inflammation. We included sE-selectin, sTM, sVCAM-1, sICAM-1, and, in 2000-2001 only, vWf as markers of endothelium dysfunction. Serum biomarkers of low-grade inflammation

(CRP, SAA, IL-6, IL-8, TNF-α) and endothelial dysfunction (sVCAM-1, sE-selectin, sTM, sICAM-1) were assessed by a multi-array detection system based on electrochemiluminescence technology (SECTOR Imager 2400, Meso Scale Discovery, Gaithersburg (MD), USA); details have been described elsewhere.[25] MPO was determined in EDTA plasma by means of sandwich ELISA (Mercodia, Uppsala, Sweden)[26] and vWF in citrated plasma by ELISA.[25]

Cognitive functioning

At the 2005–2008 examination, all participants performed an extensive neuropsychological examination including 12 verbal and non-verbal tasks, administered in a fixed order that took 90 minutes to complete. The tasks were divided into 6 cognitive domains to reduce the amount of neuropsychological variables and for clinical clarity. This division was made a priori, according to standard neuropsychological practice and cognitive theory, as described in detail in Lezak (2012). [26] The domain abstract reasoning was assessed by Raven Advanced Progressive Matrices (12-item short form). The domain memory included 4 subdomains: working memory assessed by the forward and backward digit span of the Wechsler Adult Intelligence Scale-III (WAIS-III) and the Corsi Block-Tapping Task; immediate memory and learning rate, including verbal memory assessed by the Rey Auditory Verbal Learning Test and visual memory assessed by the Location Learning Test; forgetting rate assessed by the delayed task of the Rey Auditory Verbal Learning Test and of the Location Learning Test, and incidental memory assessed by the delayed trial of the Rey-Osterrieth Complex Figure. The domain information processing speed was assessed by the Trail Making Test Part A, the Stroop Color-Word Test (parts I and II), and the subtest Digit Symbol of the WAIS-III. The domain attention and executive function was assessed by the Trail Making Test Part B, the Stroop Color-Word Test (part III), the Brixton Spatial Anticipation Test, a verbal fluency test using the N and A, and category fluency using animal names. The domain visuoconstruction was assessed by the copy trial of the Rey-Osterrieth Complex Figure. The domain language was assessed by the Token Test (short form). To estimate the premorbid level of intellectual functioning, the Dutch version of the National Adult Reading Test was used. Educational level was recorded (7 categories). Depressive symptoms were assessed with the validated

Dutch version of the 20-item Center for Epidemiologic Studies Depression Scale (CES-D; possible range 0–60). A score ≥16 was considered an indicator of possible depression.

Statistical analyses

Descriptive statistics are presented as mean ± SD or in the case of a skewed distribution as median (interquartile range). Biomarker variables with a skewed distribution (i.e. CRP, IL-6, IL-8, SAA) were log transformed. For all included biomarkers, a higher level indicates more low-grade inflammation or endothelial dysfunction. For each individual biomarker, a z-score was calculated according to the formula: (individual value - population mean)/ population standard deviation. For 2005-2008 measurements, the population means and standard deviations of 2000-2001 were used to calculate these z-scores. CRP, SAA, IL-6, IL-8, TNF-α, sICAM-1, and MPO can be interpreted as measures of the construct low-grade inflammation; sVCAM-1, sE-selectin, sTM, sICAM-1, and vWF can be interpreted as measures of the construct endothelial dysfunction. In order to reduce the influence of biological variability of each marker, to reduce measurement errors, and to prevent multiple testing problems, we calculated composite scores for each construct. This was done by averaging individual biomarker z-scores into one composite z-score for low-grade inflammation and one composite z-score for endothelial dysfunction. One biomarker, sICAM-1, was included in both overall z-scores, as it is expressed by both monocytes and the endothelium.[28] To check whether this influenced the results, we also calculated the composite z-scores for low-grade inflammation and endothelial dysfunction without sICAM-1. We also calculated z-scores for raw cognitive test scores. For each of the six cognitive domains a composite z-score was derived by averaging the z-scores of the tests comprising that domain. A sum score was calculated, representing the mean z-score over the 6 cognitive domains as a measure of overall cognitive functioning.

In the primary analysis, we examined the relation between low-grade inflammation/endothelial dysfunction and cognitive functioning. Assuming a two-sided alpha of 0.05 and 80% power, our sample size was sufficient to detect associations accounting for 1.7% explained variance (calculated using

G-power). Associations between composite z-scores of circulating biomarkers and cognitive functioning were analyzed for each cognitive domain using linear regression, with demographic variables age, sex, and estimated premorbid IQ as covariates. We calculated both prospective and cross-sectional associations, by entering low-grade inflammation and endothelial dysfunction measured in 2000-2001 or in 2005-2008 into analyses, with cognitive functioning measured in 2005-2008 as outcome. The effect of change in biomarkers (over the period of 5-7 years) on cognitive functioning was explored by adjusting the concurrent associations (all measures taken in 2005-2008) for measurements of biomarkers in 2000-2001. Because differential relations have been suggested according to sex,[15] we repeated these analyses stratified for sex. We also stratified these analyses for diabetes and hypertension status and adjusted the analyses for use of lipid-lowering and antihypertensive medication.

In secondary analyses, we further explored those cognitive domains that were significantly related to low-grade inflammation and endothelial dysfunction in the primary analysis. To investigate consistency within compound biomarker scores, we examined the relations for each individual biomarker. Furthermore, we explored the relation between low-grade inflammation/endothelial dysfunction and cognition after additional adjustments. We calculated separate linear regression models: model 1 = demographics + vascular risk factors (body mass index, current smoking, total cholesterol, presence of hypertension, and presence of diabetes mellitus), model 2 = demographics + history of cardiovascular disease, and model 3 = demographics + depressive symptoms. We then explored the size of the effects of low-grade inflammation and endothelial dysfunction on cognitive functioning, on top of the effects of these related factors. To this end, the variables of interest (vascular risk factors, cardiovascular disease, and depressive symptoms) were included in separate models in (stepwise) regression analyses, after demographics. Finally, since the association between vascular risk factors and cognitive performance has been widely established,[29] we examined whether vascular risk factors lead to cognitive decrements *via* inflammatory and endothelial processes. For this purpose, we calculated a single measure of exposure to vascular risk factors per person. This was done according to the general cardiovascular risk score of the Framingham Heart Study,[30] which is based on age, sex, HDL and total cholesterol, systolic blood pressure, anti-hypertensive treatment,

Table 1. Participant characteristics of participants with circulating biomarkers measurements in 2000-2001 and/or in 2005-2008, and with cognitive measurement in 2005-2008

	2000-2001	2005-2008
Participants (n)	363	323
Age (years)	67.6 ± 5.4	72.9 ± 5.7
Male	188 (52%)	163 (50%)
Education, median (IQR)[a,b]		4 (4-5)
Estimated premorbid IQ[a]		98 ± 13
Low-grade inflammation		
CRP (mg/L)	2.0 (0.1-147.4)	1.9 (0.1-133.1)
TNF-α (ng/L)	8.9 ± 3.1	9.1 ± 2.8
IL-6 (ng/L)	1.4 (0.4-17.7)	1.6 (0.5-30.3)
IL-8 (ng/L)	14.8 (2.5-634.7)	10.2 (3.4-668.3)
SAA (mg/L)	1.7 (0.1-220.4)	1.8 (0.1-173.7)
MPO (µg/L)	57.2 ± 16.6	Not available
sICAM-1 (µg/L)	251 ± 62	242 ± 56
Endothelial dysfunction		
vWF (%)	151 ± 52	Not available
sICAM-1 (µg/L)	251 ± 62	242 ± 56
sVCAM-1 (µg/L)	399 ± 99	403 ± 95
sTM (µg/L)	3.5 ± 0.8	3.9 ± 1.0
sE-selectin (µg/L)	18.9 ± 7.5	17.8 ± 7.1
Type 2 diabetes	61 (17%)	71 (22%)
HbA1c (%)	5.9 ± 0.6	5.8 ± 0.6
Systolic blood pressure (mmHg)	139 ± 19	146 ± 21
Diastolic blood pressure (mmHg)	82 ± 11	75 ± 11
Use of antihypertensive medication	115 (32%)	124 (38%)
Hypertension[c]	226 (62%)	229 (71%)
History of cardiovascular disease[d]	52 (14%)	51 (16%)
Total cholesterol (mmol/l)	5.7 ± 1.0	5.4 ± 1.1
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.5 ± 0.4
Use of lipid-lowering medication	61 (17%)	128 (39%)
Body mass index	27.1 ± 3.5	27.1 ± 3.6
Current smokers	42 (12%)	37 (12%)
Depressive symptoms (CES-D ≥ 16)	30 (9%)	29 (9%)

smoking, and diabetes status. To make sure that the exposure to vascular risk factors preceded the low-grade inflammation/endothelial dysfunction and cognitive decrements, we calculated the vascular risk score based on 2000-2001 measurements; low-grade inflammation and endothelial dysfunction were based on 2005-2008 measurements. The mediating effect of low-grade inflammation and endothelial dysfunction on the relation between the vascular risk score and cognitive functioning was determined by means of a bootstrapping technique.[31] Bootstrapping is a computer-based method that involves repeated sampling from the data and estimation of the mediating effect in each resampled data set. By repeating this process thousands of times, an empirical approximation of the sampling distribution is built and used to reconstruct the 95% confidence interval (CI). The mediating effect (defined as a decrease in regression coefficient B) is considered to be present if the 95% CI does not contain zero. Bootstrapped (bias-corrected) confidence intervals (5000 samples) were computed for the size of the specific mediating 'effects' using SPPS macros provided by Preacher and Hayes.[31]

RESULTS

Table 1 shows the characteristics of participants with circulating biomarkers measurements in 2000-2001 and/or in 2005-2008, and with cognitive

◀ Data are presented as means ± SD, n (%), or median (range), unless otherwise specified. IQR, interquartile range; CRP, C-reactive protein; TNF-α, tumor necrosis factor; IL-6, interleukin 6; IL-8, interleukin 8; SAA, serum amyloid A; MPO, myeloperoxidase; sICAM-1, soluble intercellular adhesion molecule 1; vWf, von Willebrand factor; sVCAM-1, soluble vascular cell adhesion molecule 1; sTM, soluble thrombomodulin; sE-selectin, soluble endothelial selectin; CES-D, Center for Epidemiologic Studies Depression Scale.

* $p \leq 0.05$ 2000-2001 measurement versus 2005-2008 measurement.

[a] Measured in 2005–2008.

[b] Seven categories.

[c] Defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or use of blood pressure-lowering medication.

[d] Defined as self-reported history of claudicatio intermittens, angina pectoris, possible myocardial infarction, amputation (Rose questionnaire[20]), stroke, or transient ischemic attack.

Table 2. Relation between low-grade inflammation and endothelial dysfunction in 2000–2001 and in 2005–2008 and cognitive functioning in 2005–2008 (left and middle column) and effect of change in biomarkers over period of 5-7 years (right column)

	Beta (95% confidence interval)		
Low-grade inflammation	**2000-2001 measurement**	**2005-2008 measurement[a]**	**Effect of change over time[b]**
Abstract reasoning	0.04 (-0.06 ; 0.14)	0.04 (-0.07 ; 0.14)	0.02 (-0.11 ; 0.14)
Memory	-0.03 (-0.13 ; 0.06)	-0.05 (-0.15 ; 0.05)	-0.06 (-0.18 ; 0.06)
Information processing speed	-0.14 (-0.23 ; -0.05)*	-0.10 (-0.19 ; -0.01)*	-0.03 (-0.14 ; 0.08)
Attention and executive functioning	-0.03 (-0.12 ; 0.06)	-0.17 (-0.27 ; -0.07)*	-0.20 (-0.31 ; -0.08)*
Visuoconstruction	-0.002 (-0.10 ; 0.10)	-0.07 (-0.18 ; 0.03)	-0.07 (-0.20 ; 0.06)
Language	-0.002 (-0.10 ; 0.10)	-0.02 (-0.11 ; 0.08)	-0.01 (-0.13 ; 0.11)
Sum score	-0.04 (-0.12 ; 0.03)	-0.08 (-0.16 ; -0.001)*	-0.07 (-0.17 ; 0.03)
Endothelial dysfunction			
Abstract reasoning	-0.04 (-0.14 ; 0.06)	0.03 (-0.08 ; 0.13)	0.06 (-0.07 ; 0.18)
Memory	-0.06 (-0.16 ; 0.03)	-0.07 (-0.17 ; 0.03)	-0.08 (-0.21 ; 0.05)
Information processing speed	-0.16 (-0.24 ; -0.07)*	-0.11 (-0.20 ; -0.01)*	-0.03 (-0.14 ; 0.09)
Attention and executive functioning	-0.12 (-0.21 ; -0.03)*	-0.14 (-0.23 ; -0.04)*	-0.10 (-0.23 ; 0.02)
Visuoconstruction	-0.08 (-0.18 ; 0.02)	-0.08 (-0.18 ; 0.03)	0.00 (-0.19 ; 0.19)
Language	-0.04 (-0.13 ; 0.05)	-0.06 (-0.15 ; 0.04)	-0.03 (-0.16 ; 0.09)
Sum score	-0.12 (-0.20 ; -0.04)*	-0.10 (-0.18 ; -0.02)*	-0.04 (-0.14 ; 0.07)

measurement in 2005-2008. Patients were on average 68 years old in 2000-2001, and 73 years in 2005-2008. In 2000-2001 and 2005-2008 respectively, 50% and 52% were men, 14% and 16% had a history of cardiovascular disease, 62% and 71% had hypertension, and 17% and 22% had type 2 diabetes. None of the participants had cognitive dysfunction severe enough to disturb day-to-day functioning.

Low-grade inflammation and endothelial dysfunction were significantly associated with age, in 2000-2001 (r=0.15 for low-grade inflammation and r=0.18 for endothelial dysfunction, both p<0.05) and in 2005-2008 (r=0.27 for low-grade inflammation and r=0.22 for endothelial dysfunction, both p<0.05). Endothelial dysfunction, but not low-grade inflammation, was also significantly associated with sex; men had more severe endothelial dysfunction than women in 2000-2001 (mean difference in z-scores: 0.17) and in 2005-2008 (mean difference in z-scores: 0.20, both p<0.05).

Associations between low-grade inflammation/ endothelial dysfunction and cognitive functioning

Low-grade inflammation and endothelial dysfunction in 2000-2001 and 2005-2008 were significantly associated with the cognitive domains information processing speed, attention and executive functioning, and the cognitive sum score in 2005-2008 (standardized betas between -0.17 and -0.03, Table 2). Low-grade inflammation and endothelial dysfunction were not related to functioning on other cognitive domains (Table 2). Increase of low-grade inflammation over time was associated with worse attention and executive functioning (beta -0.20, 95% CI -0.31 to -0.08), but not with other cognitive domains (Table 2, change in biomarkers). Change in endothelial dysfunction was not related to cognitive

◀ In all analyses, compound z-scores of circulating biomarkers and domains of cognitive functioning are used.

All analyses are adjusted for age, sex, and estimated premorbid IQ.

[a] Scores of the 2005-2008 circulating biomarker measurement are standardized on scores of the 2000-2001 measurement.

[b] The 2005-2008 circulating biomarker measurement is adjusted for the 2000-2001 measurement.

*p<0.05.

Table 3. Relation between circulating biomarkers in 2000–2001 and in 2005–2008 and cognitive functioning in 2005–2008, additional adjustments for related factors (vascular risk factors, cardiovascular disease, or depressive symptoms)

	Beta (95% confidence interval)
	Adjusted for demographics[a] (Reference; see Table 2)
Low-grade inflammation	
2000-2001 measurement	
Information processing speed	**-0.14 (-0.23 ; -0.05)***
Attention and executive functioning	-0.03 (-0.12 ; 0.06)
2005-2008 measurement	
Information processing speed	**-0.10 (-0.19 ; -0.01)***
Attention and executive functioning	**-0.17 (-0.27 ; -0.07)***
Endothelial dysfunction	
2000-2001 measurement	
Information processing speed	**-0.16 (-0.24 ; -0.07)***
Attention and executive functioning	**-0.12 (-0.21 ; -0.03)***
2005-2008 measurement	
Information processing speed	**-0.11 (-0.20 ; -0.01)***
Attention and executive functioning	**-0.14 (-0.23 ; -0.04)***

In all analyses, compound z-scores of circulating biomarkers and cognitive functioning are used.

[a] Demographics: age, sex, and estimated premorbid IQ.

[b] Vascular risk factors at the time of the circulating biomarker measurement: body mass index, current smoking, total cholesterol, hypertension (defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or use of blood pressure-lowering medication), and diabetes mellitus (defined according to WHO criteria (WHO 1999)).

	Beta (95% confidence interval)		
	Adjusted for demographics and vascular risk factors[b]	Adjusted for demographics and cardiovascular disease[c]	Adjusted for demographics and depressive symptoms[d]
	-0.15 (-0.23 ; - 0.06)*	**-0.14 (-0.23 ; -0.06)***	**-0.13 (-0.22 ; - 0.04)***
	0.00 (-0.10 ; 0.10)	-0.03 (-0.13 ; 0.06)	-0.04 (-0.13 ; 0.06)
	-0.10 (-0.19 ; -0.001)*	-0.09 (-0.18 ; 0.004)	**-0.10 (-0.19 ; -0.01)***
	-0.17 (-0.27 ; -0.07)*	**-0.14 (-0.24 ; -0.05)***	**-0.16 (-0.26 ; -0.07)***
	-0.14 (-0.23 ; - 0.05)*	**-0.15 (-0.23 ; -0.07)***	**-0.13 (-0.22 ; -0.04)***
	-0.09 (-0.19 ; 0.01)	**-0.12 (-0.21 ; -0.03)***	**-0.15 (-0.24 ; -0.05)***
	-0.09 (-0.18 ; 0.01)	**-0.10 (-0.19 ; -0.01)***	**-0.11 (-0.20 ; -0.01)***
	-0.14 (-0.24 ; -0.04)*	**-0.13 (-0.22 ; -0.04)***	**-0.13 (-0.23 ; -0.04)***

[c] Self-reported history of cardiovascular disease at the time of the circulating biomarker measurement: claudicatio intermittens, angina pectoris, possible myocardial infarction, or amputation (Rose questionnaire[20]), stroke, or transient ischemic attack.

[d] Depressive symptoms at time of the cognitive measurement: CES-D ≥ 16.

* $p<0.05$.

Table 4. Variance in cognitive functioning explained by low-grade inflammation or endothelial dysfunction, on top of demographics and related factors (vascular risk factors, cardiovascular disease, or depressive symptoms)

	R^2 change (p-value)
	Effect of circulating biomarkers on top of demographics[a]
Low-grade inflammation	
2000-2001 measurement	
Information processing speed	**0.022 (<0.001)***
Attention and executive functioning	0.001 (0.488)
2005-2008 measurement	
Information processing speed	**0.010 (0.029)***
Attention and executive functioning	**0.026 (0.001)***
Endothelial dysfunction	
2000-2001 measurement	
Information processing speed	**0.023 (<0.001)***
Attention and executive functioning	**0.014 (0.010)***
2005-2008 measurement	
Information processing speed	**0.010 (0.025)***
Attention and executive functioning	**0.018 (0.005)***

In all analyses, compound z-scores of circulating biomarkers and cognitive functioning are used.

[a] Demographics: age, sex, and estimated premorbid IQ.

[b] Vascular risk factors at the time of the circulating biomarker measurement: body mass index, current smoking, total cholesterol, hypertension (defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or use of blood pressure-lowering medication), and diabetes mellitus (defined according to WHO criteria (WHO 1999)).

	R^2 change (p-value)		
	Effect of circulating biomarkers on top of demographics and vascular risk factors[b]	**Effect of circulating biomarkers on top of demographics and cardiovascular disease[c]**	**Effect of circulating biomarkers on top of demographics and depressive symptoms[d]**
	0.023 (<0.001)*	**0.020 (0.001)***	**0.017 (0.004)***
	0.000 (0.945)	0.001 (0.467)	0.001 (0.432)
	0.006 (0.049)*	0.007 (0.061)	**0.009 (0.034)***
	0.026 (0.001)*	**0.018 (0.003)***	**0.024 (0.001)***
	0.022 (<0.001)*	**0.022 (<0.001)***	**0.016 (0.005)***
	0.008 (0.049)*	**0.014 (0.009)***	**0.020 (0.003)***
	0.006 (0.077)	**0.009 (0.030)***	**0.010 (0.026)***
	0.016 (0.008)*	**0.016 (0.007)***	**0.017 (0.006)***

[c] Self-reported history of cardiovascular disease at the time of the circulating biomarker measurement: claudicatio intermittens, angina pectoris, possible myocardial infarction, or amputation (Rose questionnaire[20]), stroke, or transient ischemic attack.

[d] Depressive symptoms at time of the cognitive measurement: CES-D ≥ 16.

* $p<0.05$.

functioning. Stratifying for sex, or for diabetes or hypertension status did not substantially change the results, neither did adjusting for use of lipid lowering and antihypertensive medication (data not shown). Excluding sICAM-1 from the composite z-scores for low-grade inflammation and endothelial dysfunction did not change the results (data not shown).

Secondary analyses

In secondary analyses, associations between the biomarkers and the cognitive domains information processing speed and attention and executive functioning were studied in more detail. First, effect sizes of individual biomarkers of low-grade inflammation and endothelial dysfunction for both cognitive domains ranged from -0.004 to -0.19. No single biomarker could be identified that drives the association with cognitive functioning, rather it is likely that all biomarkers contribute to the effect (see Supplementary Material). Second, adjusting for vascular risk factors or for cardiovascular disease at the time of biomarker measurement somewhat attenuated the relations between the biomarkers and cognitive functioning (Table 3). However, the majority of the associations remained statistically significant, indicating that the relation is partly independent of vascular factors. Additional adjustments for depressive symptoms did not change the results (Table 3), which indicates that the relation between low-grade inflammation/endothelial dysfunction and cognitive functioning is independent of depressive symptoms. Third, stepwise regression analyses showed that age, sex, and estimated premorbid IQ together explained 37% of the variance for information processing speed and 26% for attention and executive functioning (both $p<0.05$). Low-grade inflammation or endothelial dysfunction additionally explained up to 2.6% of the variance in cognitive functioning (Table 4). The explained variance of low-grade inflammation or endothelial dysfunction remained similar when vascular risk factors, cardiovascular disease, or depressive symptoms were included in the model (Table 4). This further indicates that the effects were independent of these related factors. Finally, an individual vascular risk score based on vascular risk factors in 2000-2001 was significantly related to cognitive performance in 2005-2008 (information processing speed: B -3.57, 95% CI -5.05 to -2.08; attention and executive functioning: B -1.35, 95% CI -2.51 to -0.19). Bootstrapping

analyses showed that this relation was significantly mediated by low-grade inflammation (for information processing speed: decrease in B -0.35, 95% CI -0.80 to -0.08; for attention and executive functioning: decrease in B -0.35, 95% CI -0.84 to -0.06) and endothelial dysfunction (for information processing speed: decrease in B -0.48, 95% CI -1.11 to -0.13; for attention and executive functioning: decrease in B -0.37, 95% CI -0.88 to -0.08). This suggests that low-grade inflammation and endothelial dysfunction are intermediate factors in the relation between vascular risk factors and cognitive functioning.

DISCUSSION

The present study shows that in a population-based sample of older persons, circulating biomarkers of low-grade inflammation and endothelial dysfunction were associated with decrements in cognitive functioning on the domains information processing speed, and attention and executive functioning.

This is the first time that the relation between low-grade inflammation and endothelial dysfunction and cognitive functioning is examined with composite scores of multiple circulating biomarkers in combination with extensive neuropsychological examination. Previous studies in the general population investigated the relation between individual circulating biomarkers of inflammation and cognitive impairment or cognitive decline (for a recent review, see Bettcher et al. (2013)[16]). Generally, studies reported that elevated levels of CRP, IL-6, IL-8, and TNF-α were associated with poorer cognitive functioning, for example[32–35], but also see studies that did not find an association: [36–38]. One study found an association between sICAM-1 and cognitive decline.[39] We identified only two previous studies on the associations between SAA or MPO and cognitive functioning, which both did not find statistically significant results.[38,40] Compared to low-grade inflammation, relatively fewer studies have addressed endothelial dysfunction in relation to cognitive functioning. Some studies on vWF found an association with cognitive functioning or dementia,[41,42] whereas others did not.[43–45] The cognitive domains that are found to be related to low-grade inflammation or endothelial dysfunction vary. In fact, associations between these markers and cognitive functioning have been reported for virtually all main cognitive domains:

memory, executive functioning, processing speed, attention, and global cognitive scores. Furthermore, some studies report an association with one domain and not with another, whereas other studies find reversed results.[16] In the current study, associations were found for the domains information processing speed and attention and executive functioning: the cognitive domains that are thought to be related to global brain functions. No associations were found for memory, abstract reasoning, language, or visuoconstruction, which are more closely related to localized cortical functions. This distinction may imply that low-grade inflammation and endothelial dysfunction are related to cognitive impairment via diffuse, global brain damage as has been reported before in populations with vascular damage.[46] This would also fit with a model in which low-grade inflammation and endothelial dysfunction interact with vascular factors to contribute to cognitive decrements (Figure). This is supported by the mediating role of low-grade inflammation and endothelial dysfunction in the relation between vascular risk factors and cognitive functioning.

However, vascular factors are not the whole story. Associations between low-grade inflammation or endothelial dysfunction and cognitive functioning were in part statistically independent of vascular risk factors and cardiovascular disease. Likewise, the 1-3% explained variance in cognitive functioning that was accounted for by low-grade inflammation or endothelial dysfunction was not altered after adding vascular risk factors or cardiovascular disease to the model. A possible explanation for these findings is that life-time exposure to vascular risk, starting from middle age, is of greater importance for the association with low-grade inflammation, endothelial dysfunction, and cognitive functioning, than a late-life risk factor profile.[47] Moreover, the processes of low-grade inflammation and endothelial dysfunction by themselves may account for some of the variance in cognitive functioning, on top of vascular factors. Several possible mechanisms have been suggested for this relation. First, inflammatory cytokines may sort a direct effect on cognitive functioning due to their role in neurotransmitter and neuroendocrine responses.[4] Second, low-grade inflammation and endothelial dysfunction are related to the development of neurodegenerative diseases such as AD.[48,49] It should be noted, however, that these processes may also be downstream of vascular factors and are thus unlikely to affect cognitive functioning in isolation. Importantly, the associations

between low-grade inflammation and endothelial dysfunction and cognitive functioning were rather weak. Apparently, other causative factors play a role in determining cognitive outcome, such as cognitive reserve or neurodegenerative pathology. Additional explanations for the weak association are biological variability and measurement error of the biomarkers.

Low-grade inflammation and endothelial dysfunction are also involved in the pathobiology of depressive symptoms,[50,51] which, in turn, are related to cognitive functioning.[11] Specifically, executive dysfunction may impact treatment response in late-life depression.[51] Therefore, we specifically examined the role of depressive symptoms. In the present study, associations between low-grade inflammation or endothelial dysfunction and cognitive functioning were independent of depressive symptoms. It should be noted, however, that depressive symptoms were at a subclinical level in the current sample.

Strengths of the present study include the detailed recording of vascular and metabolic determinants and cognitive functioning over an extended period as well as a detailed neuropsychological assessment, in a well-defined population-based cohort of older participants. Our study also has some limitations. First, the study had an observational design and neuropsychological assessment was performed only once. Therefore, no inferences about causality can be made. Further studies with longitudinal neuropsychological data are needed to explore the temporal relations between low-grade inflammation, endothelial dysfunction and cognitive performance, within the vascular cascade. Second, a possible selection bias may have occurred due to attrition of this elderly population during follow-up, since persons with a less favorable vascular risk factor profile at baseline in 1989 or with severe cognitive dysfunction are more likely to be lost to follow-up. Third, an assumption in the construction of the z-scores for low-grade inflammation and endothelial dysfunction is that its components are equally important, which is not necessarily true. Still, the majority of the individual biomarkers contributed to the effect, suggesting that the constructs of low-grade inflammation and endothelial dysfunction had biological validity. Fourth, in the analysis of the effects of change in low-grade inflammation and endothelial dysfunction on cognitive performance, the influence of biological variability and measurement error of the biomarkers was present for both time points. Therefore, the analysis may be less reliable and the presented effect may

be an underestimation. Fifth, we had no detailed information available on the use of immunosuppressive agents, or periodic use of NSAIDs. However, the use of immunosuppressants at the population level is low and the relation between NSAIDs and cognitive decline in observational studies is unclear.[53] Besides their inflammation suppressing effects, these medications are related to sickness behavior, which is related to worse cognitive performance.[4] Thus, if anything, the use of immunosuppressive agents would weaken the association between low-grade inflammation and cognitive dysfunctioning and the presented effect may be an underestimation. Finally, we did not measure brain inflammatory responses directly. Peripheral inflammation indices only provide a proxy for potential cerebral processes. Nonetheless, we are not aware of methods that directly measure brain inflammatory responses, and that can be used at the population level.

In conclusion, biomarkers of low-grade inflammation and endothelial dysfunction are related to impairment on cognitive domains that depend on global information processing, and play an intermediate role in the association between vascular risk factors and cognitive functioning. This association fits into a model in which low-grade inflammation and endothelial dysfunction, together with vascular risk factors and cardiovascular disease, contribute to cognitive decline in aging.

REFERENCES

1. Knopman DS, Roberts R. Vascular risk factors: imaging and neuropathologic correlates. *Journal of Alzheimer's disease*. 2010;20:699–709.
2. Gorelick PB. Role of inflammation in cognitive impairment: results of observational epidemiological studies and clinical trials. *Annals of the New York Academy of Sciences.* 2010;1207:155–62.
3. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. *Basic research in cardiology*. 2008;103:398–406.
4. Wilson CJ, Finch CE, Cohen HJ. Cytokines and cognition--the case for a head-to-toe inflammatory paradigm. *Journal of the American Geriatrics Society.* 2002;50:2041–56.
5. Esposito K, Giugliano D. The metabolic syndrome and inflammation: association or causation? *Nutrition, metabolism, and cardiovascular diseases*. 2004;14:228–32.
6. Dharmashankar K, Widlansky ME. Vascular endothelial function and hypertension: insights and directions. *Current hypertension reports*. 2010;12:448–55.
7. Granger DN, Rodrigues SF, Yildirim A, Senchenkova EY. Microvascular responses to cardiovascular risk factors. *Microcirculation*. 2010;17:192–205.
8. Cosentino F, Volpe M. Hypertension, stroke, and endothelium. *Current hypertension reports*. 2005;7:68–71.
9. Galea J, Brough D. The role of inflammation and interleukin-1 in acute cerebrovascular disease. *Journal of inflammation research*. 2013;6:121–128.
10. Alexopoulos GS, Morimoto SS. The inflammation hypothesis in geriatric depression. *International journal of geriatric psychiatry*. 2011;26:1109–18.
11. Thomas AJ, O'Brien JT. Depression and cognition in older adults. *Current opinion in psychiatry*. 2008;21:8–13.
12. Taylor WD, Aizenstein HJ, Alexopoulos GS. The vascular depression hypothesis: mechanisms linking vascular disease with depression. *Molecular psychiatry*. 2013;:1–12.
13. Kuo H-K, Yen C-J, Chang C-H, Kuo C-K, Chen J-H, Sorond F. Relation of C-reactive protein to stroke, cognitive disorders, and depression in the general population: systematic review and meta-analysis. *Lancet neurology*. 2005;4:371–80.
14. Quinn TJ, Gallacher J, Deary IJ, Lowe GDO, Fenton C, Stott DJ. Association between circulating hemostatic measures and dementia or cognitive impairment: systematic review and meta-analyzes. *Journal of thrombosis and haemostasis*. 2011;9:1475–82.

15. Hedges DW, Farrer TJ, Brown BL. Association between C-reactive protein and cognitive deficits in elderly men and women: a meta-analysis. *International psychogeriatrics*. 2012;24:1387–92.
16. Bettcher BM, Kramer JH. Inflammation and clinical presentation in neurodegenerative disease: a volatile relationship. *Neurocase*. 2013;19:182–200.
17. Mooy JM, Grootenhuis PA, de Vries H, Valkenburg HA, Bouter LM, Kostense PJ, Heine RJ. Prevalence and determinants of glucose intolerance in a Dutch caucasian population. The Hoorn Study. *Diabetes care*. 1995;18:1270–3.
18. De Vegt F, Dekker JM, Jager A, Hienkens E, Kostense PJ, Stehouwer CDA, Nijpels G, Bouter LM, Heine RJ. Relation of impaired fasting and postload glucose with incident type 2 diabetes in a Dutch population: The Hoorn Study. *Journal of the American Medical Association*. 2001;285:2109–13.
19. Van den Berg E, Dekker JM, Nijpels G, Kessels RPC, Kappelle LJ, de Haan EHF, Heine RJ, Stehouwer CDA, Biessels GJ. Cognitive functioning in elderly persons with type 2 diabetes and metabolic syndrome: the Hoorn study. *Dementia and geriatric cognitive disorders*. 2008;26:261–9.
20. Rose G, Blackburn H, Gillum R. *Cardiovascular Survey Methods*. Geneva, WHO Press. 1982.
21. Ross R. Atherosclerosis--an inflammatory disease. *The New England journal of medicine*. 1999;340:115–26.
22. Borissoff JI, Spronk HMH, ten Cate H. The hemostatic system as a modulator of atherosclerosis. *The New England journal of medicine*. 2011;364:1746–60.
23. Aird WC. Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. *Circulation research*. 2007;100:158–73.
24. Schalkwijk CG, Stehouwer CDA. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. *Clinical science*. 2005;109:143–59.
25. Van Bussel BCT, Henry RM a, Schalkwijk CG, Dekker JM, Nijpels G, Stehouwer CD a. Low-grade inflammation, but not endothelial dysfunction, is associated with greater carotid stiffness in the elderly: the Hoorn Study. *Journal of hypertension*. 2012;30:744–52.
26. Van der Zwan LP, Scheffer PG, Dekker JM, Stehouwer CDA, Heine RJ, Teerlink T. Hyperglycemia and oxidative stress strengthen the association between myeloperoxidase and blood pressure. *Hypertension*. 2010;55:1366–72.
27. Lezak MD, Howieson DB, Bigler ED, Tranel D. *Neuropsychological assessment*. 5th ed. Oxford University Press; 2012.

28. Schram MT, Stehouwer CDA. Endothelial dysfunction, cellular adhesion molecules and the metabolic syndrome. *Hormone and metabolic research*. 2005;37 Suppl 1:49–55.
29. Van den Berg E, Kloppenborg RP, Kessels RPC, Kappelle LJ, Biessels GJ. Type 2 diabetes mellitus, hypertension, dyslipidemia and obesity: A systematic comparison of their impact on cognition. *Biochimica et biophysica acta*. 2009;1792:470–81.
30. D'Agostino RB, Vasan RS, Pencina MJ, Wolf P a, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation*. 2008;117:743–53.
31. Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behavior Research Methods*. 2008;40:879–891.
32. Yaffe K, Lindquist K, Penninx BW, Simonsick EM, Pahor M, Kritchevsky S, Launer L, Kuller L, Rubin S, Harris T. Inflammatory markers and cognition in well-functioning African-American and white elders. *Neurology*. 2003;61:76–80.
33. Noble JM, Manly JJ, Schupf N, Tang MX, Mayeux R, Luchsinger JA. Association of C-reactive protein with cognitive impairment. *Archives of neurology*. 2010;67:87–92.
34. Teunissen CE, Boxtel MPJ Van, Bosma H, Bosmans E, Delanghe J, Bruijn C De. Inflammation markers in relation to cognition in a healthy aging population. *Journal of Neuroimmunology*. 2003;134:142–150.
35. Baune BT, Ponath G, Golledge J, Varga G, Arolt V, Rothermundt M, Berger K. Association between IL-8 cytokine and cognitive performance in an elderly general population--the MEMO-Study. *Neurobiology of aging*. 2008;29:937–44.
36. Alley DE, Crimmins EM, Karlamangla A, Hu P, Seeman TE. Inflammation and rate of cognitive change in high-functioning older adults. The journals of gerontology. Series A, *Biological sciences and medical sciences*. 2008;63:50–5.
37. Dik MG, Jonker C, Hack CE, Smit JH, Comijs HC, Eikelenboom P. Serum inflammatory proteins and cognitive decline in older persons. *Neurology*. 2005;64:1371–1377.
38. Jefferson AL, Massaro JM, Beiser AS, Seshadri S, Larson MG, Wolf PA, Au R, Benjamin EJ. Inflammatory markers and neuropsychological functioning: the Framingham Heart Study. *Neuroepidemiology*. 2011;37:21–30.

39. Rafnsson SB, Deary IJ, Smith FB, Whiteman MC, Rumley A, Lowe GDO, Fowkes FGR. Cognitive decline and markers of inflammation and hemostasis: the Edinburgh Artery Study. *Journal of the American Geriatrics Society*. 2007;55:700–7.
40. Jordanova V, Stewart R, Davies E, Sherwood R, Prince M. Markers of inflammation and cognitive decline in an African-Caribbean population. *International journal of geriatric psychiatry*. 2007;22:966–73.
41. Mari D, Parnetti L, Coppola R, Bottasso B, Reboldi GP, Senin U, Mannucci PM. Hemostasis abnormalities in patients with vascular dementia and Alzheimer's disease. *Thrombosis and haemostasis*. 1996;75:216–8.
42. Stott DJ, Spilg E, Campbell a M, Rumley A, Mansoor M a, Lowe GD. Haemostasis in ischaemic stroke and vascular dementia. *Blood coagulation & fibrinolysis*. 2001;12:651–7.
43. Barber M, Tait RC, Scott J, Rumley A, Lowe GDO, Stott DJ. Dementia in subjects with atrial fibrillation: hemostatic function and the role of anticoagulation. *Journal of thrombosis and haemostasis*. 2004;2:1873–8.
44. Carcaillon L, Gaussem P, Ducimetière P, Giroud M, Ritchie K, Dartigues JF, Scarabin PY. Elevated plasma fibrin D-dimer as a risk factor for vascular dementia: the Three-City cohort study. *Journal of thrombosis and haemostasis*. 2009;7:1972–8.
45. Gallacher J, Bayer A, Lowe G, Fish M, Pickering J, Pedro S, Dunstan F, White J, Yarnell J, Ben-Shlomo Y. Is sticky blood bad for the brain?: Hemostatic and inflammatory systems and dementia in the Caerphilly Prospective Study. *Arteriosclerosis, thrombosis, and vascular biology*. 2010;30:599–604.
46. Prins ND, van Dijk EJ, den Heijer T, Vermeer SE, Jolles J, Koudstaal PJ, Hofman A, Breteler MMB. Cerebral small-vessel disease and decline in information processing speed, executive function and memory. *Brain*. 2005;128:2034–41.
47. Reijmer YD, Biessels GJ. Vascular risk scores for dementia: age matters. *Archives of neurology*. 2011;68:267; author reply 268–70.
48. Grammas P. Neurovascular dysfunction, inflammation and endothelial activation: implications for the pathogenesis of Alzheimer's disease. *Journal of neuroinflammation*. 2011;8:26.
49. Ray S, Britschgi M, Herbert C, Takeda-Uchimura Y, Boxer A, Blennow K, Friedman LF, Galasko DR, Jutel M, Karydas A, Kaye J a, Leszek J, Miller BL, Minthon L, Quinn JF, Rabinovici GD, Robinson WH, Sabbagh MN, So YT, Sparks DL, Tabaton M, Tinklenberg J, Yesavage J a, Tibshirani R, Wyss-Coray

T. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. *Nature medicine*. 2007;13:1359–62.

50. Krishnadas R, Cavanagh J. Depression: an inflammatory illness? *Journal of neurology, neurosurgery, and psychiatry*. 2012;83:495–502.
51. Van Sloten TT, Schram MT, Adriaanse MC, Dekker JM, Nijpels G, Teerlink T, Scheffer PG, Pouwer F, Schalkwijk CG, Stehouwer CDA, Henry RMA. Endothelial dysfunction is associated with a greater depressive symptom score in a general elderly population: the Hoorn Study. *Psychological medicine*. 2013;:1–14.
52. Pimontel MA, Culang-Reinlieb ME, Morimoto SS, Sneed JR. Executive dysfunction and treatment response in late-life depression. *International journal of geriatric psychiatry*. 2012;27:893–9.
53. Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, Petersen RC, Schneider JA, Tzourio C, Arnett DK, Bennett DA, Chui HC, Higashida RT, Lindquist R, Nilsson PM, Roman GC, Sellke FW, Seshadri S. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42:2672–713.

Supplementary Material Relation between all individual biomarkers (measured in 2000-2001 and 2005-2008) and cognitive domains of information processing speed and attention and executive functioning (measured in 2005-2008).

	Beta (95% confidence interval)	
	Information processing speed in 2005-2008	
Low-grade inflammation	**2000-2001**	**2005-2008**
CRP (mg/L)	**-0.12 (-0.21 ; -0.04)***	**-0.09 (-0.18 ; -0.003)***
TNF-α (ng/L)	**-0.10 (-0.19 ; -0.02)***	-0.08 (-0.17 ; 0.01)
IL-6 (ng/L)	**-0.09 (-0.17 ; -0.01)***	-0.06 (-0.15 ; 0.04)
IL-8 (ng/L)	-0.04 (-0.12 ; 0.05)	-0.004 (-0.11 ; -0.10)
SAA (mg/L)	-0.07 (-0.16 ; 0.01)	-0.07 (-0.16 ; 0.02)
MPO (µg/L)	-0.08 (-0.16 ; 0.01)	MPO not available
sICAM-1 (µg/L)	**-0.14 (-0.22 ; -0.06)***	**-0.09 (-0.18 ; -0.001)***
Endothelial dysfunction	**2000-2001**	**2005-2008**
vWF (%)	**-0.13 (-0.21 ; -.05)***	VWf not available
sICAM-1 (µg/L)	**-0.14 (-0.22 ; -0.06)***	**-0.09 (-0.18 ; -0.001)***
sVCAM-1 (µg/L)	**-0.10 (-0.18 ; -0.01)***	-0.09 (-0.18 ; 0.01)
sTM (µg/L)	-0.05 (-0.13 ; 0.04)	-0.08 (-0.17 ; -0.01)
sE-selectin (µg/L)	-0.07 (-0.16 ; 0.01)	-0.02 (-0.12 ; 0.07)

In all analyses, compound z-scores of domains of cognitive functioning are used.

All analyses are adjusted for age, sex, and estimated premorbid IQ.

[a] The 2005-2008 circulating biomarker measurement is adjusted for the 2000-2001 measurement.

[b] Scores of the 2005-2008 circulating biomarker measurement are standardized on scores of the 2000-2001 measurement.

*p<0.05.

Beta (95% confidence interval)	
Attention and executive functioning in 2005-2008	
2000-2001	**2005-2008**
-0.02 (-0.11 ; 0.08)	**-0.14 (-0.23 ; -0.04)***
-0.05 (-0.14 ; 0.04)	**-0.18 (-0.27 ; -0.08)***
0.04 (-0.06 ; 0.13)	-0.08 (-0.18 ; 0.02)
-0.02 (-0.11 ; 0.07)	-0.02 (-0.11 ; 0.07)
0.01 (-0.07 ; 0.10)	**-0.12 (-0.22 ; -0.03)***
0.002 (-0.06 ; 0.06)	MPO not available
-0.10 (-0.19 ; -0.01)*	**-0.16 (-0.21 ; -0.02)***
2000-2001	**2005-2008**
-0.03 (-0.12 ; 0.06)	vWf not available
-0.10 (-0.19 ; -0.01)*	**-0.16 (-0.24 ; -0.02)***
-0.12 (-0.22 ; -0.03)*	**-0.19 (-0.28 ; -0.09)***
-0.01 (-0.11 ; 0.08)	-0.07 (-0.16 ; 0.03)
-0.11 (-0.21 ; -0.02)*	-0.01 (-0.10 ; 0.08)

CHAPTER 5

VASCULAR RETINOPATHY IN RELATION TO COGNITIVE FUNCTIONING IN AN OLDER POPULATION – THE HOORN STUDY

SM Heringa, I Walraven, AC Moll, E van den Berg, G Nijpels, CDA Stehouwer, YD Reijmer, LJ Kappelle, JM Dekker, GJ Biessels

Accepted for publication in revised form as a letter to the editor in:
Journal of the American Geriatrics Association

ABSTRACT

BACKGROUND: The retinal and cerebral vasculature share anatomical and physiological characteristics and may be vulnerable to similar disease processes. As such, retinal vascular abnormalities, which are easy to visualize, may be a marker of late-life cognitive impairment of vascular origin.

OBJECTIVES: To assess the association between vascular retinopathy and cognitive functioning in older non-demented persons.

DESIGN: Cross-sectional population-based study.

SETTING: The Hoorn Study, a community-based cohort study of cardiovascular risk.

PARTICIPANTS: Three hundred thirteen participants without dementia (mean age 72.9±5.6 years).

MEASUREMENTS: All participants underwent fundus photography and extensive neuropsychological examination. Fundus photographs were graded as no, any or severe vascular retinopathy. Raw test scores of six cognitive domains (abstract reasoning, memory, information processing speed, attention and executive functioning, visuoconstruction, and language) were standardized into z-scores per test and subsequently averaged per domain. A z-sum score, reflecting global cognitive functioning, was also calculated by averaging the z-scores of the different domains.

RESULTS: Any vascular retinopathy was diagnosed in 74 participants (23.6%), of whom six had severe vascular retinopathy. We found no differences in cognitive functioning between participants with no and with any vascular retinopathy (mean differences in z- sum scores: 0.07 (95% confidence interval: -0.05 to 0.18, p=0.25) or between participants with no and with severe vascular retinopathy (mean differences in z-sum scores: -0.01 (95% confidence interval: -0.37 to 0.34, p=0.94).

CONCLUSION: At the population level, vascular retinopathy is not a marker of reduced cognitive performance in older individuals.

INTRODUCTION

Cognitive impairment and dementia are important health problems that may be caused by vascular damage in the brain.[1] The cerebral vasculature is anatomically, embryologically, and physiologically related to that of the retina, and both are sensitive to exposure to vascular risk factors.[2,3] Vascular damage to the retina is easy to measure noninvasively. Hence, visualization of retinal vessels may offer insight into the status of the vessels in the brain, and thus provide insight into vascular causes of late-life cognitive impairment.

A recent systematic review showed a small but consistent relation between retinal vascular changes and dementia or cognitive functioning, that is most pronounced in persons with relatively severe retinal vascular abnormalities.[4] Community-dwelling older persons with vascular retinopathy have an increased risk for cognitive impairment or dementia, although associations with various cognitive domains in persons without dementia are inconsistent.[4,5] We aimed to extend these findings in an older population who underwent detailed evaluation of the retinal vasculature and had a comprehensive neuropsychological assessment. We examined whether people with signs of vascular retinopathy had worse cognitive functioning than those without.

METHODS

Participants

313 participants (mean age 72.9±5.6 years, 52% male) from the population-based Hoorn cohort were included in this cross-sectional study. Detailed information on the Hoorn Study has been reported previously.[6,7] All participants underwent ophthalmological and neuropsychological examination between 2005 and 2008. The Hoorn Study was approved by the medical ethics committee of the VU University Medical Center and was performed in accordance with the guidelines of the Helsinki Declaration. Written informed consent was obtained from all participants.

Measurements

Participants underwent physical examination, including measurements of body mass index (calculated as weight divided by squared height), and assessment of

blood pressure, glycated hemoglobin level (HbA1c, %), and total cholesterol. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or use of blood pressure-lowering medication, presence of type 2 diabetes mellitus was based on WHO criteria (WHO 1999). History of cardiovascular disease was defined as self-reported intermittent claudication, angina pectoris, possible myocardial infarction, amputation, stroke, or transient ischemic attack.

Ophthalmological examination

The retina was examined with fundus photography after mydriasis. Methods were similar to those reported previously in the Hoorn Study.[8] All photographs were graded by an experienced ophthalmologist. Any vascular retinopathy was defined as the presence in at least one eye of hypertensive vessel changes, sclerotic vessel changes, hemorrhages and microaneurysms, preretinal hemorrhages, vitreous hemorrhages, flame shaped hemorrhages, hard exudates, cotton wool spots, intraretinal microvascular abnormalities, venous beading, areas of neovascularization, fibrous proliferation, laser coagulation scars, focal arteriolar narrowing, arteriovenous nicking, venous or arteriolar occlusion, arterial narrowing, retinal vasculopathy, atherosclerosis, or a clinical diagnosis of hypertensive retinopathy or diabetic retinopathy (based on EURODIAB [9]). Of these signs of any vascular retinopathy, a subgroup was defined as signs of severe vascular retinopathy: presence of hemorrhages and microaneurysms, cotton wool spots, intraretinal microvascular abnormalities, areas of neovascularization, fibrous proliferation, laser coagulation scars, or a clinical diagnosis of severe diabetic retinopathy (EURODIAB[9] grade 3, 4, or 5).

Cognitive assessment

All participants underwent an extensive neuropsychological examination measuring 6 cognitive domains: abstract reasoning, memory, information processing speed, attention and executive function, visuoconstruction, and language.[7] Raw test scores were standardized into z-scores per test and subsequently averaged per domain. A sum score was also calculated by averaging the z-scores of the six domains. The Dutch version of the National Adult Reading Test was used as an estimation of the premorbid level of

intellectual functioning. Educational level was recorded (7 categories). Depressive symptoms were assessed with the validated Dutch version of the 20-item Center for Epidemiologic Studies Depression Scale (CES-D; possible range 0–60). A score ≥16 was considered an indicator of possible depression.

Statistical analysis

Between-group differences in population characteristics between participants with and without any vascular retinopathy were analyzed with analysis of

Table 1. Characteristics of participants

	Vascular retinopathy	No vascular retinopathy	P-value
Participants	76 (24%)	239 (76%)	
Age (years)	74.1 ± 5.6	72.5 ± 5.6	0.03*
Male	32 (43%)	131 (55%)	0.054
Education, median (IQR)[a]	4 (4 – 5)	4 (4 – 5)	0.26
Estimated premorbid IQ	98 ± 13	98 ± 13	0.97
Type 2 diabetes	16 (22%)	49 (21%)	0.49
Hypertension[b]	55 (74%)	168 (70%)	0.30
HbA1c (%)	6.0 ± 0.6	5.7 ± 0.6	<0.01*
Total cholesterol (mmol/L)	5.4 ± 1.1	5.3 ± 1.1	0.78
Systolic blood pressure (mmHg)	149 ± 19	146 ± 21	0.22
Diastolic blood pressure (mmHg)	74 ± 9	75 ± 11	0.47
Body mass index	28 ± 4	27 ± 4	0.12
History of cardiovascular disease[c]	16 (22%)	40 (17%)	0.21
Current smokers	13 (18%)	31 (13%)	0.08
Depressive symptoms (CES-D ≥ 16)	9 (12%)	20 (8%)	0.22

Data are presented as means ± SD, n (%), or median (range), unless otherwise specified.

[a] Seven categories.

[b] Defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or use of blood pressure-lowering medication.

[c] Defined as self-reported history of claudicatio intermittens, angina pectoris, possible myocardial infarction, amputation (Rose questionnaire[10]), stroke, or transient ischemic attack.

variance for continuous variables, Mann-Whitney U tests for non-parametric data and chi-square tests for proportions. Between-group differences in cognitive functioning were examined with analysis of covariance, with sex, age, and estimated premorbid IQ as covariates. In separate analyses, we added as covariates hypertension, systolic blood pressure, diabetes, cardiovascular disease, or depression. Furthermore, we performed sensitivity analyses by comparing persons with and without severe vascular retinopathy.

RESULTS

Any vascular retinopathy in at least one eye was present in 74 participants (23.6%). This group included persons with a clinical diagnosis of hypertensive retinopathy (59 participants, 18.8% of the sample), atherosclerosis (n=15, 4.8%), venous occlusion (n=2, 0.6%) and/or diabetic retinopathy (n=3, 1.0%). The most common retinopathy sign was sclerotic vessel changes (n=15, 4.8%). Other signs were: arteriovenous nicking (n=4, 1.3%), venous occlusion (n=3, 1.0%), or microaneurysms (n=2, 0.6%). Hard exudates, venous beading, and focal narrowing were each present in one individual (0.3%). None of the participants had cotton wool spots, intraretinal microvascular abnormalities, areas of neovascularization, fibrous proliferation, arteriolar occlusion, photocoagulation scars, preretinal hemorrhages, vitreous hemorrhages, flame shaped hemorrhages, or narrow arteries.

Population characteristics are shown in Table 1. Participants with any vascular retinopathy were older than those without (74.1±5.6 versus 72.5±5.6 years), were less likely to be male (43% versus 57%), and had higher HbA1c levels (6.0±0.6% versus 5.7±0.6%). None of the participants had cognitive dysfunction severe enough to disturb day-to-day functioning.

There were no differences in cognitive functioning between participants with and without any vascular retinopathy (Table 2). Mean differences in z-scores ranged from -0.02 to 0.13, all $p>0.05$. Additional adjustment for depressive symptoms, hypertension, systolic blood pressure, cardiovascular disease, or diabetes did not change the results (data not shown).

Six participants (1.9%) had severe vascular retinopathy. They were slightly older than participants without vascular retinopathy (77.0±7.1 versus 72.5±5.6

years, p=0.052). Cognitive functioning did not differ significantly from those without vascular retinopathy. Mean differences in z-scores ranged from -0.24 to 0.12, all p>0.05.

Table 2. Comparison of cognitive functioning between participants with and without any or severe vascular retinopathy (no retinopathy = reference)

	Mean difference in z-scores, beta with 95% CI	
	Any (n=74) versus no (n=239) vascular retinopathy	Severe (n=6) versus no (n=239) vascular retinopathy
Abstract reasoning	0.07 (-0.18 to 0.32)	-0.02 (-0.77 to 0.81)
Memory	0.09 (-0.03 to 0.21)	0.12 (-0.24 to 0.47)
Information processing speed	-0.02 (-0.21 to 0.17)	-0.10 (-0.68 to 0.49)
Attention and executive functioning	0.02 (-0.13 to 0.17)	0.01 (-0.42 to 0.45)
Visuoconstruction	0.10 (-0.15 to 0.36)	-0.24 (-1.05 to 0.57)
Language	0.13 (-0.09 to 0.35)	0.01 (-0.68 to 0.71)
Sum score	0.07 (-0.05 to 0.18)	-0.01 (-0.37 to 0.34)

DISCUSSION

The present study shows that in a population-based sample of older persons participating in the Hoorn Study, presence of any or severe vascular retinopathy was unrelated to cognitive functioning.

Our study sample permitted us to detect differences between persons with and without any vascular retinopathy with effect sizes as small as ~0.30 (power 0.80, alpha 0.05). Thus, statistical power was sufficient to detect decrements in cognitive performance that are relevant for daily functioning.[11] The observed differences between participants with and without any vascular retinopathy were very small, with point estimates for effect sizes below 0.15 with narrow 95% confidence intervals. This indicates that the absence of an association was probably true and not caused by low statistical power. For severe vascular

retinopathy, on the other hand, statistical power was considerably lower because of the small number of participants showing this pathology. Therefore, no definite conclusions about the relation between severe vascular retinopathy and cognitive functioning can be drawn based on our data. Further studies in larger cohorts are needed to address whether this extreme end of the spectrum of retinal vascular damage is associated with impaired cognition.

Thus far, a limited number of studies have investigated the relation between vascular retinopathy and cognitive functioning in the general population, as has been reviewed recently.[4] Generally, neuropsychological examinations in these studies were less elaborate than in the current study. Effect sizes for any vascular retinopathy were in the range of -0.25 to 0.03, which is in line with the present results. Taking into account a possible publication bias, even more studies may have been performed that failed to obtain significant associations. Taken together, these findings show that in non-demented individuals in the general older population, there is no clinically relevant relation between the presence of any vascular retinopathy and cognitive performance. Nevertheless, people with any vascular retinopathy are at risk for performing at the very low end of the spectrum of cognitive functioning, in particular dementia.[4,5]

Strengths of the present study include the detailed recording of vascular and retinal determinants and extensive neuropsychological assessment in a well-defined population-based cohort of older participants. Our study also has some limitations. First, because of the observational cross-sectional design, it could not be determined whether vascular retinopathy may predict cognitive decline in the years to come. Second, visual impairment caused by retinal vascular changes may have confounded performance on cognitive tests.[12] Visual acuity was not formally tested, therefore we cannot rule out the possibility that persons with vascular retinopathy have encountered difficulties during cognitive assessment due to poorer vision. However, only persons with sufficient vision to complete the neuropsychological examination were included in this study.

In conclusion, in our population-based sample of older individuals, any or severe vascular retinopathy were unrelated to cognitive functioning. These findings indicate that in the general population, vascular retinopathy is not a marker of reduced cognitive performance in older individuals.

REFERENCES

1. Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, Petersen RC, Schneider JA, Tzourio C, Arnett DK, Bennett DA, Chui HC, Higashida RT, Lindquist R, Nilsson PM, Roman GC, Sellke FW, Seshadri S. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42:2672–713.
2. Wong TY, Klein R, Klein BE, Tielsch JM, Hubbard L, Nieto FJ. Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. *Survey of ophthalmology*. 2001;46:59–80.
3. Wong TY, McIntosh R. Systemic associations of retinal microvascular signs: a review of recent population-based studies. *Ophthalmic & physiological optics*. 2005;25:195–204.
4. Heringa SM, Bouvy WH, van den Berg E, Moll AC, Kappelle LJ, Biessels GJ. Associations between retinal microvascular changes and dementia, cognitive functioning, and brain imaging abnormalities: a systematic review. *Journal of cerebral blood flow and metabolism*. 2013;33:983–95.
5. Schrijvers EMC, Buitendijk GHS, Ikram MK, Koudstaal PJ, Hofman A, Vingerling JR, Breteler MMB. Retinopathy and risk of dementia: the Rotterdam Study. *Neurology*. 2012;79:365–70.
6. Mooy JM, Grootenhuis PA, de Vries H, Valkenburg HA, Bouter LM, Kostense PJ, Heine RJ. Prevalence and determinants of glucose intolerance in a Dutch caucasian population. The Hoorn Study. *Diabetes care*. 1995;18:1270–3.
7. Van den Berg E, Dekker JM, Nijpels G, Kessels RPC, Kappelle LJ, de Haan EHF, Heine RJ, Stehouwer CDA, Biessels GJ. Cognitive functioning in elderly persons with type 2 diabetes and metabolic syndrome: the Hoorn study. *Dementia and geriatric cognitive disorders*. 2008;26:261–9.
8. Van Leiden HA, Dekker JM, Moll AC, Nijpels G, Heine RJ, Bouter LM, Stehouwer CDA, Polak BCP. Blood pressure, lipids, and obesity are associated with retinopathy: the hoorn study. *Diabetes care*. 2002;25:1320–5.
9. Aldington SJ, Kohner EM, Meuer S, Klein R, Sjølie AK. Methodology for retinal photography and assessment of diabetic retinopathy: the EURODIAB IDDM complications study. *Diabetologia*. 1995;38:437–44.

10. Rose G, Blackburn H, Gillum R. *Cardiovascular Survey Methods*. Geneva, WHO Press. 1982.
11. Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd ed. New York: Academic Press; 1988.
12. Farragher J, Jassal SV. Relationship Between Retinopathy and Cognitive Impairment May Be Confounded by Visual Impairment. *American journal of kidney diseases*. 2013;61:1042.

PART 2

CEREBRAL MARKERS OF MICROVASCULAR DISEASE AND COGNITIVE FUNCTIONING

CHAPTER 6

MULTIPLE MICROBLEEDS ARE RELATED TO CEREBRAL NETWORK DISRUPTIONS IN PATIENTS WITH EARLY ALZHEIMER'S DISEASE

SM Heringa, YD Reijmer, A Leemans, HL Koek, LJ Kappelle, GJ Biessels; on behalf of the Utrecht Vascular Cognitive Impairment (VCI) Study Group

Published in: Journal of Alzheimer's Disease 2014;38:211-21.

ABSTRACT

BACKGROUND: Cerebral microbleeds are a manifestation of small vessel disease and are common in patients with Alzheimer's disease (AD). However, their clinical significance in this condition is uncertain. We hypothesized that microbleeds contribute to disturbances of the cerebral network in AD and as such may affect cognition.

OBJECTIVE: To examine the relation between microbleeds and brain networks in patients with amnestic mild cognitive impairment (aMCI) or early AD.

METHODS: Sixty-seven patients (77.9±7.5 years) with aMCI (n=29) or early AD (n=38) underwent cognitive testing and 3Tesla MRI. Microbleeds were rated visually. Diffusion tensor imaging and graph theoretical analysis were used to reconstruct brain networks and to quantify network efficiency for each patient. Network measures were compared between patients without and with ≥1 microbleeds and between patients without or with ≥3 microbleeds. In secondary analyses, cognitive functioning was compared between groups. Analyses were adjusted for age and sex, and additionally for other markers of small vessel disease and atrophy.

RESULTS: Network measures did not differ between patients with ≥1 microbleed (n=26) and patients without microbleeds (n=41). However, patients with ≥3 microbleeds (n=11) showed significant white matter disruptions, longer path length and less global efficiency than patients without microbleeds, independent of other markers of small vessel disease and atrophy. Cognitive functioning did not differ between patients without microbleeds and patients with ≥1 or ≥3 microbleeds.

CONCLUSION: Multiple microbleeds are related to structural network disruption in patients with early AD, but their direct impact on cognitive functioning appears to be limited.

INTRODUCTION

Cerebral microbleeds are a manifestation of cerebral small vessel diseases.[1] Microbleeds are visible as focal hypointense lesions on MRI sequences sensitive to magnetic susceptibility[1,2] and are common in patients with Alzheimer's disease (AD).[3] Several studies have demonstrated an association between the presence of both lobar and deep microbleeds and impaired cognition in healthy older people,[4–7] and in patients with small vessel disease,[8] stroke,[9–11] or vascular dementia.[12,13] In patients with AD, however, results are inconclusive: most studies have not found an association between presence of microbleeds and concurrent Mini-Mental State Examination (MMSE) scores[14–19] or decline in MMSE scores,[20] whereas only one study found worse cognitive functioning in patients with >8 microbleeds.[21]

The observed relations between microbleeds and cognitive impairment may have several explanations. Microbleeds may directly impact cognitive functioning, due to disturbances of brain structure. Alternatively, microbleeds are not a causative factor themselves, but rather represent the presence of other vascular processes in the brain that underlie the cognitive deficits. The two main types of vascular pathology that are considered to cause microbleeds are hypertensive vasculopathy and cerebral amyloid angiopathy (CAA).[1] Presence of microbleeds may thus reflect more extensive brain damage in the context of vascular risk factors and/or CAA and associated parenchymal AD type pathologies. It is therefore conceivable that microbleeds or microbleed-related pathologies affect cognitive functioning by disrupting white matter brain connectivity, leading to less efficient transfer of information between brain regions. However, whether presence of microbleeds is indeed related to disturbed integrity of the brain network is yet unknown. Recently, it has become possible to reconstruct brain networks using diffusion MRI.[22,23] The quality or efficiency of the network can then be quantified using graph theoretical analysis.[24] Following this approach, previous studies have shown that the brain network is affected in patients with mild cognitive impairment (MCI),[25] AD,[26] or with a vascular risk factor such as diabetes mellitus.[27] Furthermore, the quality of the network has shown to be strongly related to cognitive function in patients with early AD.[28]

In the present study, we explore the relation between microbleeds and the integrity of the cerebral network in patients with MCI or dementia due to AD. We hypothesize that microbleeds are associated with less efficient brain networks and as such could affect cognitive functioning.

METHODS

Participants

Sixty-seven patients (mean age 77.9 ± 7.5 years, 44% male), 38 with early-stage AD dementia and 29 with amnestic MCI (aMCI; considered to represent a transitional stage between normal aging and AD[29]) were recruited via the memory clinic at the University Medical Center Utrecht. All participants underwent clinical examination, cognitive testing and a 3 Tesla MRI scan. Probable or possible AD was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria.[30] AMCI was diagnosed according to the Petersen criteria.[29] Exclusion criteria for the present study were a history of stroke in the last 2 years, a history of stroke with subsequent cognitive deterioration, schizophrenia or other psychotic disorders, major depression, alcohol abuse, brain tumor, epilepsy or encephalitis. Patients with a more advanced stage of AD, indicated by a clinical dementia rating >1[31] or a MMSE score <20,[32] were also excluded. The clinical dementia rating score was determined by the patients' doctor and the diagnosis (AD/aMCI) was established at a multidisciplinary meeting.

The study was approved by the medical ethics committee of the University Medical Center Utrecht, the Netherlands and was conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all participants.

MRI data acquisition

MRI data were acquired on a Philips 3.0 Tesla scanner using a standardized protocol and consisted of a T2*-weighted scan (48 continuous slices, reconstructed voxel size: 0.99x0.99x3.00 mm^3), a 3D T1 scan (192 continuous slices, reconstructed voxel size: 1.00x1.00x1.00 mm^3), a fluid attenuated

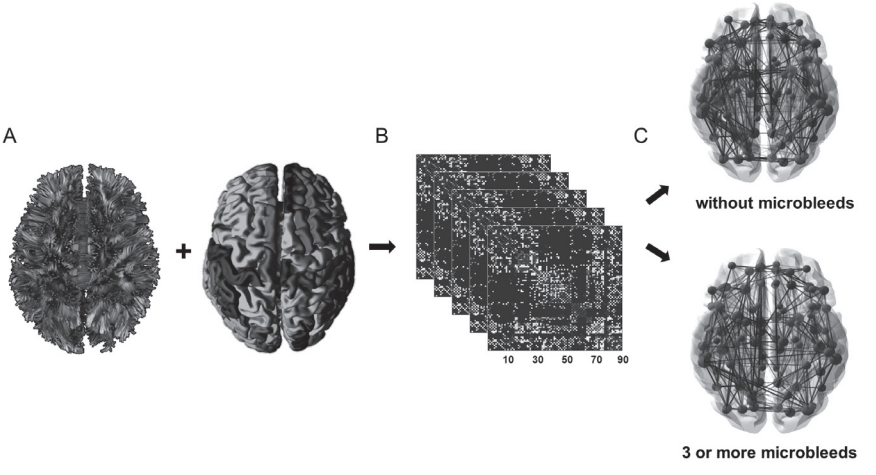
inversion recovery (FLAIR) scan (48 continuous slices, reconstructed voxel size: 0.96x0.95x3 mm^3), and diffusion-weighted MRI data using a single-shot spin echo EPI sequence (48 contiguous slices, acquired isotropic voxel size 2.50 mm, 45 isotropically distributed diffusion-sensitizing gradients with a b-value of 1200 s/mm^2, and one b=0 s/mm^2).[33]

DTI-processing and network reconstruction

Diffusion tensor imaging (DTI) pre-processing and reconstruction of the white matter network included several steps. 1) The diffusion MRI data were corrected for subject motion and eddy current distortions[34] using ExploreDTI (www.exploredti.com)[35] as described previously.[36] 2) For each voxel the fractional anisotropy (FA) and mean diffusivity (MD) were computed. 3) For each dataset, whole-brain fiber tractography was performed using a deterministic streamline approach.[37] The whole-brain fiber tract reconstructions were parcellated using the automated anatomical labeling atlas (AAL).[38] Applying this procedure, we obtained 90 cortical and subcortical regions (with the cerebellum excluded). Each region of interest (ROI) of the AAL template represented a node of the network. Two AAL nodes were considered to be connected if a fiber pathway was present with two end points located in these regions. Using this approach, a binary 90 x 90 connectivity matrix was obtained for each subject. Each connection edge was scaled by the total number of fiber trajectories composing that connection, resulting in a weighted connectivity matrix for each individual[23,28] (Figure 1).

We investigated the properties of the structural network using the Brain Connectivity Toolbox.[39] For each weighted network connectivity matrix, measures of local interconnectivity (i.e., clustering coefficient and local efficiency) and global connectivity (i.e., characteristic shortest path length and global efficiency) were obtained.[39] The *clustering coefficient* quantifies the extent to which neighboring brain regions are connected with each other. The *local efficiency* reflects the average efficiency of local clusters, an indicator of fault tolerance of the network. The *characteristic shortest path length* quantifies the average number of connections between regions along the shortest paths. A shorter path length reflects a higher *global efficiency* of the network: a measure of parallel information processing ability.

In addition to the weighted measures, we calculated the normalized measures of the binary network by dividing each measure by the values obtained from 100 matched random networks.[40,41] Further details and in-depth discussion about these measures can be found in Rubinov et al.[39]



A) Whole-brain fiber tract reconstructions were parcellated using a standard template consisting of 90 cortical and subcortical brain regions. B) Using this procedure, a weighted 90 x 90 connectivity matrix was obtained for each participant. These matrices were used to compute measures of local and global connectivity. C) Visualization of the brain network, averaged per group of patients without microbleeds or with multiple microbleeds. Not all connections are visualized.

Panel A reused with permission from [27], copyright of the American Diabetes Association.

Microbleeds, other markers of small vessel disease, and atrophy on MRI

Number and location of microbleeds were rated visually on the T2*-weighted scans by two raters, blinded to clinical information, according to the Microbleed Anatomical Rating Scale.[42] Ratings of the two observers were compared and in case of discordance they performed a consensus rating or an experienced third rater was consulted.

Other measures of small vessel disease and atrophy were also assessed, for use as covariates in the primary analyses. White matter hyperintensity load,

lacunar and cortical infarcts were assessed on FLAIR images, with the T1 as reference. Total white matter hyperintensity load was assessed with the Age Related White Matter Changes (ARWMC) scale[43] and was quantified as the sum score of all brain regions (range 0-30). Lacunar infarcts were defined as lesions <1.5 cm in diameter, with a hypointense core on T1 and FLAIR images and with an appearance unlike a perivascular space. Numbers of lacunar infarcts were recorded. Medial temporal lobe atrophy (MTA) was rated on coronal reconstructions of T1 images, using a 5-point rating scale (0-4).[44] Scores were averaged across both hemispheres. Intraclass correlation coefficients (ICC) for the ratings were 0.79 for microbleed number, 0.83 for white matter hyperintensities, 0.77 for lacunar infarcts, and 0.69 for medial temporal atrophy score.

Total brain volume was calculated using the freely available FreeSurfer software, which yields ml and percentage of intracranial volume. To limit the number of analyses, we calculated composite scores for other markers of small vessel disease and atrophy. First, raw scores were converted into z-scores. The z-scores white matter hyperintensities and lacunar infarcts were averaged into the composite score "small vessel disease", the z-scores for medial temporal atrophy and total brain volume were averaged into the composite score "atrophy".

Cognitive testing

All participants underwent a standardized cognitive assessment. 'Executive functioning' was assessed by the Trail Making Test - Part B,[45] the Stroop Color-Word Test card III,[46] and a Verbal Fluency Test. 'Verbal memory' was assessed by the immediate and delayed task of the Rey Auditory Verbal Learning Test.47 'Information processing speed' was assessed by the Trail Making Test part A,[45] the Stroop Color-Word Test card I and II,[46] and the Letter Digit Substitution Test.[48] For each domain, the raw test scores were standardized into z-scores and averaged to obtain one composite z-score per cognitive domain.

Statistical analysis

Patient characteristics were analyzed with analysis of variance for continuous variables, Mann- Whitney U tests for non-parametric data and $\chi 2$ tests for proportions. Comparisons were made between patients with no versus any microbleeds. After post-hoc inspection of the microbleed frequency

distribution, we enhanced contrast by comparing patients with no versus three or more microbleeds. Primary outcome measures were characteristics of the cerebral network (characteristic shortest path length, clustering coefficient, global efficiency, local efficiency). Indicators of white matter microstructure, whole-brain FA and MD, are important for DTI-based cerebral network reconstruction, and were therefore also compared between groups. Additionally, we assessed the relation between the actual microbleed number and network measures within the group of patients with microbleeds. This was done with Spearman correlations between microbleed number and individualized age and sex-adjusted residual z-scores for the different network measures.

Secondary outcome measures were domain scores of cognitive functioning: executive functioning, verbal memory, and information processing speed. Between-group differences were analyzed with analysis of covariance. Analyses with white matter microstructure and network outcome measures were adjusted for age and sex, analyses with cognitive outcome measures were adjusted for age, sex, and education level. Additional models included adjustments for compound scores of small vessel disease and atrophy.

RESULTS

Group characteristics are shown in Table 1. Patients with microbleeds (n=26, 39%) were older and had more white matter hyperintensities than patients without microbleeds (n=41). Patients with 3 or more microbleeds (n=11, 16%) additionally had more often lacunar infarcts.

A total of 134 microbleeds were found. Most of these (95; 71%) were located in lobar regions, versus 19 (14%) in deep and 21 (16%) in infratentorial regions. Of the 26 patients with 1 or more microbleed, 12 (46%) had strictly lobar, 6 (23%) had strictly deep/infratentorial, and 8 (31%) had both lobar and deep/infratentorial microbleeds. All 11 patients with 3 or more microbleeds had lobar microbleeds, of whom 5 (46%) had strictly lobar microbleeds. The structural brain networks of all patients had a much higher level of local clustering ($\gamma >> 1$, mean 1.96±0.30, range 1.32 to 3.17) and an equivalent characteristic shortest path length between any pair of nodes ($\lambda \sim 1$, mean 1.08±0.02, range 1.03 to 1.13), compared to random networks, which is typical for a so-called small-world architecture.

Table 1. Group characteristics

	Without microbleeds	With one or more microbleeds	With three or more microbleeds
N	41	26	11
Age (years)	76.4±7.3	80.7±6.9*	81.7±5.0*
Male sex	16 (39%)	12 (46%)	6 (55%)
Education level (1-7) (median (IQR))	4 (4-6)	5 (4-6)	5 (5-7)
MMSE (0-30)	25.3±2.8	25.1±2.4	25.1±2.9
Systolic blood pressure (mmHg)	147±19	157±25	155±27
White matter hyperintensity load (0-30)	7.3±4.6	10.8±4.4*	11.8±5*
Lacunar infarcts (present; median (range))	14 (34%); 0 (0-13)	15 (58%); 0 (0.5-9)	9 (82%); 2 (0-8)*
Cortical infarcts (present)	3 (7%)	4 (16%)	2 (18%)
Total brain volume (ml)	940±100	945±94	941±93
Medial temporal lobe atrophy (0-4)	1.5±1.0	1.7±0.8	2.0±0.7
Diagnosis of AD	22 (54%)	16 (42%)	6 (55%)

* significantly different from group without microbleeds ($p<0.05$)

Data are presented as means ± standard deviations or n (%), unless otherwise specified.

Relation between microbleeds and characteristics of the cerebral network

First, comparisons were made between patients without microbleeds and patients with one or more microbleeds. The groups did not differ significantly with regard to local or global network connectivity, nor with regard to microstructural white matter characteristics (i.e., FA and MD values) (all $p>0.05$; see Table 2 for unadjusted means). Adjusting for other markers of small vessel disease and atrophy or for clinical diagnosis (aMCI or AD) did not change these results (data not shown). Comparing patients with lobar microbleeds versus patients without lobar microbleeds yielded similar results (data not shown). Numbers of patients with subcortical microbleeds were too small to perform statistical analyses.

Subsequently, comparisons were made between patients without microbleeds and patients with three or more microbleeds. Patients with multiple

microbleeds showed significant longer path length, and less global and local network efficiency compared with patients without microbleeds (see Table 2 for unadjusted means; see left panel of Table 3 (Model 1) for age and sex-adjusted mean differences in z-scores). In patients with three or more microbleeds, path length and global efficiency remained significantly different when normalized binary networks measures were examined, i.e. measures that are independent of network strength (path length (λ): 1.10±0.02 versus 1.08±0.02; global efficiency: 0.95±0.01 versus 0.96±0.01; both $p<0.05$). Normalized local efficiency based on binary networks did not differ significantly between groups (1.54±0.10 versus 1.43±0.23, $p>0.05$).

Within the group of patients with one or more microbleeds, the number of microbleeds was associated with all network parameters: path length (Spearman's $r=0.452$), clustering coefficient ($r=-0.446$) global efficiency ($r=-0.525$), and local efficiency ($r=-0.455$), all $p<0.05$) (see Figure 2 for correlation plots). Patients with multiple microbleeds also showed significant lower FA values and higher MD values, reflecting a reduced coherence in white matter tract organization (see Table 2). Other markers of small vessel disease and atrophy were also related to some of the network measures (Table 3, right panel). Nevertheless, adjusting the differences in network measures between patients with three or more and

Table 2. White matter microstructure and network parameters in patients with and without microbleeds

	Without microbleeds (n=41)	With one or more microbleeds (n=26)	With three or more microbleeds (n=11)
Characteristic shortest path length	4.281±0.588	4.561±0.616	4.912±0.496
Clustering coefficient	6.769±1.030	6.568±1.081	6.073±0.775
Global network efficiency	0.306±0.030	0.293±0.033	0.274±0.024
Local network efficiency	2.670±0.221	2.598±0.264	2.447±0.220
Whole-brain FA	0.458±0.020	0.448±0.020	0.435±0.014
Whole-brain MD[a]	0.804±0.047	0.836±0.051	0.874±0.043

Data are presented as means ± standard deviations (unadjusted).

[a]MD values x 10-3 mm2/s

patients without microbleeds for these other imaging abnormalities did not change the pattern of the results (left panel of Table 3 (Model 2)). Adjusting for clinical diagnosis did not change the results (data not shown).

Relation between microbleeds and cognitive functioning

Cognitive functioning did not differ on any of the three cognitive domains between patients without microbleeds and patients with one or more microbleeds (all p>0.05 for unadjusted mean z-scores), or between patients

Figure 2. Relation between number of microbleeds and network parameters within patients with microbleeds

Network z-scores are adjusted for age and sex on an individual basis

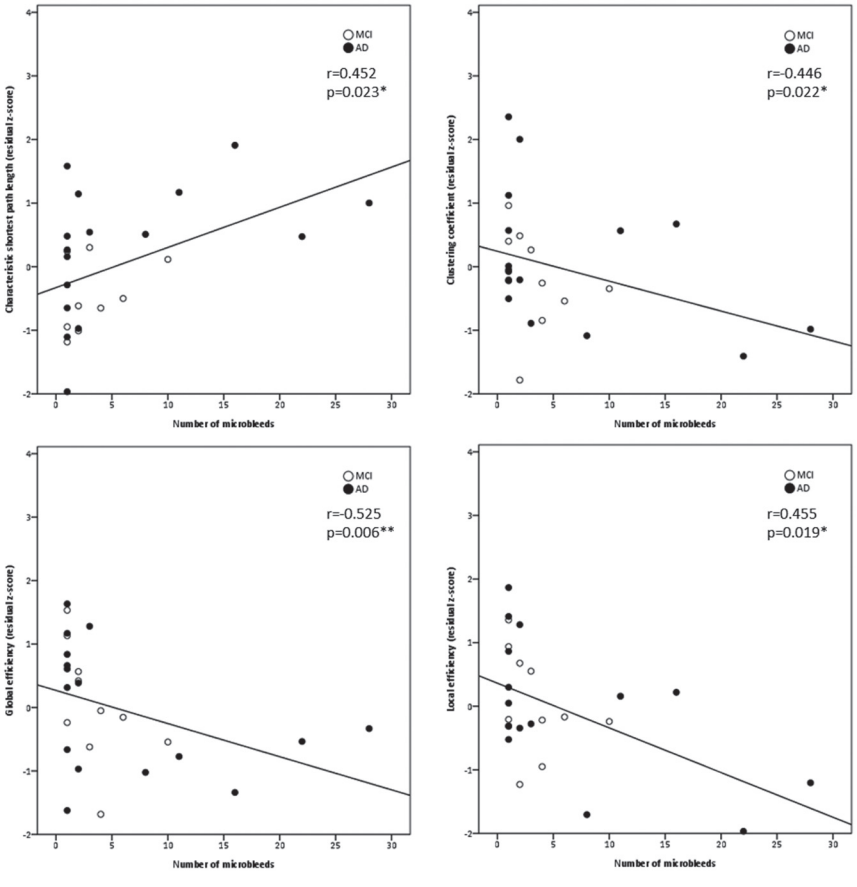


Table 3. Relation between microbleeds, other measures of small vessel disease and atrophy and network parameters

	Mean difference in z-scores (95%CI) between patients without versus with three or more microbleeds	
	Model 1	**Model 2**
Characteristic shortest path length	0.90 (0.19 ; 1.61)*	0.82 (0.17 ; 1.48)*
Clustering coefficient	-0.43 (-1.06 ; 0.21)	-0.16 (-0.81 ; 0.49)
Global network efficiency	-1.00 (-1.67 ; -0.33)**	-0.95 (-1.57 ; -0.33)**
Local network efficiency	-0.70 (-1.33 ; -0.07)*	-0.43 (-1.06 ; 0.21)
Whole-brain FA	-1.05 (-1.73 ; -0.37)**	-0.58 (-1.12 ; -0.05)*
Whole-brain MDc	1.10 (0.46 ; 1.74)**	0.60 (0.12 ; 1.08)*

* p<0.05, ** p<0.01. [a]Analyses adjusted for age and sex. [b]More brain volume means less atrophy. cMD values x 10-3 mm2/s.

Model 1: adjusted for age and sex

Table 4. Cognitive functioning in patients with and without microbleeds

	Means ± standard deviations[a]		
	Without microbleeds (n=41)	**With one or more micro-bleeds (n=26)**	**With three or more micro-bleeds (n=11)**
Executive functioning	-0.013±0.948	0.021±1.097	-0.216±0.961
Verbal memory	0.005±0.999	-0.008±1.021	0.141±1.076
Information processing speed	0.036±0.903	-0.059±1.159	-0.041±1.482

Patients without microbleeds did not differ from patients with one or more microbleeds on measures of white matter microstructure or network parameters (all p>0.05), mean differences are provided only for the comparison between patients with no versus three or more microbleeds.

Standardized regression coefficients (95%CI) for other measures of small vessel disease and atrophy[a]

Lacunar infarcts (number)	White matter hyperintensity load (0-30)	Medial temporal lobe atrophy (0-4)	Brain volume (% of intracranial volume)[b]
0.48 (0.25 ; 0.72)**	0.37 (0.12 ; 0.61)**	-0.04 (-0.29 ; 0.23)	-0.27 (-0.54 ; 0.01)
-0.23 (-0.48 ; 0.02)	-0.23 (-0.48 ; 0.01)	-0.37 (-0.60 ; -0.13)**	0.14 (-0.14 ; 0.41)
-0.51 (-0.75 ; -0.27)**	-0.31 (-0.56 ; -0.05)*	0.09 (-0.17 ; 0.36)	-0.14 (-0.15 ; 0.43)
-0.39 (-0.63 ; -0.16)**	-0.29 (-0.54 ; -0.05)*	-0.35 (-0.58 ; -0.11)**	0.22 (-0.06 ; 0.49)
-0.75 (-0.93 ; -0.56)**	-0.54 (-0.76 ; -0.32)**	-0.19 (-0.45 ; 0.06)	0.18 (-0.10 ; 0.47)
0.67 (0.50 ; 0.86)**	0.58 (0.38 ; 0.78)**	0.37 (0.14 ; 0.59)**	-0.50 (-0.73 ; -0.26)**

Model 2: additionally adjusted for other markers of small vessel disease (compound score of white matter hyperintensity load and lacunar infarcts) and atrophy (compound score of total brain volume and medial temporal atrophy score)

6

Mean difference in z-scores (95%CI) between patients without microbleeds versus three or more microbleeds

Model 1	Model 2
-0.03 (-0.71 ; 0.66)	0.13 (-0.59 ; 0.86)
0.32 (-0.39 ; 1.02)	0.53 (-0.23 ; 1.29)
-0.04 (-0.85 ; 0.76)	0.10 (-0.72 ; 0.91)

[a]Unadjusted data

Model 1: adjusted for age, sex, and education level

Model 2: additionally adjusted for other markers of small vessel disease (compound score of white matter hyperintensity load and lacunar infarcts), and atrophy (compound score of total brain volume and medial temporal atrophy score)

without microbleeds and patients with three or more microbleeds (all p>0.05 for unadjusted mean z-scores and mean differences in z-scores) (Table 3). Adjusting for other markers of small vessel disease and atrophy did not change these results (data not shown).

DISCUSSION

This is the first study that examined microbleeds in relation to structural cerebral network deficits in patients with early AD. Our results show that multiple microbleeds were associated with disruptions of the microstructure and organization of the cerebral network, independent of other measures of small vessel disease or atrophy. However, microbleeds were not associated with cognitive dysfunctioning.

The human cerebral network has a so-called small-world architecture that allows for an efficient transfer and integration of information between brain areas.[24] "Small-world-ness" is defined as a relative high local clustering ($\gamma > 1$) and approximate equivalent characteristic path length ($\lambda \sim 1$) compared with random networks, and has been previously reported in healthy subjects.[23,49] Patients in our study exhibited such a typical small-world architecture, showing that the white matter networks were constructed reliably. The efficiency of structural brain networks as assessed with DTI declines with age[50] and is affected in patients with aMCI or AD.[25,26] Disruptions in local or global connectivity in patients with AD have also been shown with other approaches, such as cortical thickness measurements,[51,52] and functional imaging techniques, i.e. EEG,[53,54] MEG,[55] or fMRI. [56–59] The causes of these network disruptions in patients with early AD are not yet clear,[60] but likely involve pathological changes in both gray and white matter. The present study shows that multiple microbleeds or processes related to the presence of microbleeds are likely to contribute to these disruptions, independent of atrophy or other measures of small vessel disease.

Cerebral network characteristics are related to cognitive functioning in patients with early AD.[26] In a previous study on our patient cohort we have reported that this association is largely independent of white matter hyperintensities, infarcts, and atrophy.[28] In the present study, multiple microbleeds were related to the cerebral network, but not to cognitive functioning. The latter finding is in line with previous literature.[14–18,20] Only one

previous study reported an association with cognitive impairment in patients with AD, but this concerned patients with eight or more microbleeds.[21] On the other hand, in non-demented individuals from population-based cohorts both the presence and number of microbleeds are associated with impaired cognition.[4–7] These findings may imply that microbleeds are primarily a marker of presence of brain pathology underlying cognitive impairment, but not so much of its severity. Thus, several pathological processes, including multiple microbleeds and related pathologies, may affect the cerebral network and together determine cognitive outcome in early AD.

Several pathophysiological mechanisms may underlie the association between microbleeds and network disruption. Firstly, the possibility that microbleeds themselves play a causal role in network disruptions cannot be rejected. However, the relatively large alterations in whole-brain diffusion measures we observed in patients with multiple microbleeds suggest more widespread white matter pathology, likely to be of vascular origin. In our study, white matter lesions and lacunar infarcts could not fully explain the association between microbleeds and network disruptions, suggesting that other forms of white matter damage are involved that are not captured by the classical imaging markers of small vessel disease. Alternatively, CAA-related pathologies, in particular amyloid in the vessel walls[61] or parenchyma[62] may be responsible for the observed microstructural changes and network disturbances. Because we did not examine the presence of amyloid in the current study, we cannot confirm or refute this hypothesis. Additionally, in patients with early AD, cerebral atrophy is a possible factor contributing to network disruptions.[63] The current observed association between microbleeds and network disturbances was independent of atrophy. Future studies are needed to explore the role of these processes in network disruptions.

Strengths of the present study include the comprehensive scan protocol, including high quality clinical diffusion MRI data, and the assessment of multiple cognitive domains in a well-defined cohort of patients with early AD. Our study also has some limitations. In this explorative study, multiple outcome measures were examined in a relatively small population. This may have increased the risk for type I and type II errors. Secondly, due to a limited number of patients with numerous microbleeds, we could not determine reliably whether very

high microbleed counts were associated with cognitive dysfunctioning, as was suggested by previous findings.[21] A third limitation is that we could not examine the relation between network disturbances and cognitive functioning within the group of patients with microbleeds, again due to an insufficient number of patients. Fourth, our sample of patients with early AD was not homogeneous because patients were included with clinical diagnoses of MCI or possible AD. Furthermore, the cross-sectional design does not allow for conclusions about causality in the relation between microbleeds and network disruptions. Finally, given that the majority of the white matter contains complex fiber architecture64 and that DTI measures and tractography results are heavily affected by such "crossing fiber" configurations, it is possible that DTI based network analyses may be affected in a complicated way.[65]

In conclusion, multiple microbleeds are related to structural network disruption in patients with early AD, but this appears to be accompanied by more pronounced cognitive dysfunction.

REFERENCES

1. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, Launer LJ, Van Buchem MA, Breteler MM. Cerebral microbleeds: a guide to detection and interpretation. *Lancet neurology*. 2009;8:165–74.
2. Werring DJ, Gregoire SM, Cipolotti L. Cerebral microbleeds and vascular cognitive impairment. *Journal of the neurological sciences*. 2010;299:131–5.
3. Cavalieri M, Schmidt H, Schmidt R. Structural MRI in normal aging and Alzheimer's disease: white and black spots. *Neuro-degenerative diseases*. 2012;10:253–6.
4. Poels MMF, Ikram MA, van der Lugt A, Hofman A, Niessen WJ, Krestin GP, Breteler MMB, Vernooij MW. Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. *Neurology*. 2012;78:326–33.
5. Yakushiji Y, Nishiyama M, Yakushiji S, Hirotsu T, Uchino A, Nakajima J, Eriguchi M, Nanri Y, Hara M, Horikawa E, Kuroda Y. Brain microbleeds and global cognitive function in adults without neurological disorder. *Stroke*. 2008;39:3323–8.
6. Yakushiji Y, Noguchi T, Hara M, Nishihara M, Eriguchi M, Nanri Y, Nishiyama M, Hirotsu T, Nakajima J, Kuroda Y, Hara H. Distributional impact of brain microbleeds on global cognitive function in adults without neurological disorder. *Stroke*. 2012;43:1800–5.
7. Takashima Y, Mori T, Hashimoto M, Kinukawa N, Uchino A, Yuzuriha T, Yao H. Clinical correlating factors and cognitive function in community-dwelling healthy subjects with cerebral microbleeds. *Journal of stroke and cerebrovascular diseases*. 2011;20:105–10.
8. Van Norden AGW, van den Berg HAC, de Laat KF, Gons RAR, van Dijk EJ, de Leeuw F-E. Frontal and temporal microbleeds are related to cognitive function: the Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Cohort (RUN DMC) Study. *Stroke*. 2011;42:3382–6.
9. Werring DJ, Frazer DW, Coward LJ, Losseff NA, Watt H, Cipolotti L, Brown MM, Jäger HR. Cognitive dysfunction in patients with cerebral microbleeds on T2*-weighted gradient-echo MRI. *Brain*. 2004;127:2265–75.
10. Mok V, Wong KK, Xiong Y, Wong A, Schmidt R, Chu W, Hu X, Leung EYL, Chen S, Chen Y, Tang WK, Chen X, Ho CL, Wong KS, Wong STC. Cortical and frontal atrophy are associated with cognitive impairment in age-related confluent white-matter lesion. *Journal of neurology, neurosurgery, and psychiatry*. 2011;82:52–7.

11. Gregoire SM, Scheffler G, Jäger HR, Yousry TA, Brown MM, Kallis C, Cipolotti L, Werring DJ. Strictly lobar microbleeds are associated with executive impairment in patients with ischemic stroke or transient ischemic attack. *Stroke; a journal of cerebral circulation*. 2013;44:1267–72.
12. Nardone R, De Blasi P, Seidl M, Höller Y, Caleri F, Tezzon F, Ladurner G, Golaszewski S, Trinka E. Cognitive function and cholinergic transmission in patients with subcortical vascular dementia and microbleeds: a TMS study. *Journal of neural transmission*. 2011;118:1349–58.
13. Seo SW, Hwa Lee B, Kim E-J, Chin J, Sun Cho Y, Yoon U, Na DL. Clinical significance of microbleeds in subcortical vascular dementia. *Stroke*. 2007;38:1949–51.
14. Pettersen JA, Sathiyamoorthy G, Gao F-Q, Szilagyi G, Nadkarni NK, St George-Hyslop P, Rogaeva E, Black SE. Microbleed topography, leukoaraiosis, and cognition in probable Alzheimer disease from the Sunnybrook dementia study. *Archives of neurology*. 2008;65:790–5.
15. Cordonnier C, van der Flier WM, Sluimer JD, Leys D, Barkhof F, Scheltens P. Prevalence and severity of microbleeds in a memory clinic setting. *Neurology*. 2006;66:1356–60.
16. Hanyu H, Tanaka Y, Shimizu S, Takasaki M, Abe K. Cerebral microbleeds in Alzheimer's disease. *Journal of neurology*. 2003;250:1496–7.
17. Nakata Y, Shiga K, Yoshikawa K, Mizuno T, Mori S, Yamada K, Nakajima K. Subclinical brain hemorrhages in Alzheimer's disease: evaluation by magnetic resonance T2*-weighted images. *Annals of the New York Academy of Sciences*. 2002;977:169–72.
18. Nakata-Kudo Y, Mizuno T, Yamada K, Shiga K, Yoshikawa K, Mori S, Nishimura T, Nakajima K, Nakagawa M. Microbleeds in Alzheimer disease are more related to cerebral amyloid angiopathy than cerebrovascular disease. *Dementia and geriatric cognitive disorders*. 2006;22:8–14.
19. Ayaz M, Boikov AS, Haacke EM, Kido DK, Kirsch WM. Imaging cerebral microbleeds using susceptibility weighted imaging: one step toward detecting vascular dementia. *Journal of magnetic resonance imaging*. 2010;31:142–8.
20. Van der Vlies AE, Goos JDC, Barkhof F, Scheltens P, van der Flier WM. Microbleeds do not affect rate of cognitive decline in Alzheimer disease. *Neurology*. 2012;79:763–9.

21. Goos JDC, Kester MI, Barkhof F, Klein M, Blankenstein MA, Scheltens P, van der Flier WM. Patients with Alzheimer disease with multiple microbleeds: relation with cerebrospinal fluid biomarkers and cognition. *Stroke*. 2009;40:3455–60.
22. Tournier J-D, Mori S, Leemans A. Diffusion tensor imaging and beyond. *Magnetic Resonance in Medicine*. 2011;65:1532–56.
23. Hagmann P, Cammoun L, Gigandet X, Meuli R, Honey CJ, Wedeen VJ, Sporns O. Mapping the structural core of human cerebral cortex. *PLoS biology*. 2008;6:e159.
24. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. Nature reviews. *Neuroscience*. 2009;10:186–98.
25. Shu N, Liang Y, Li H, Zhang J, Li X, Wang L, He Y, Wang Y, Zhang Z. Disrupted topological organization in white matter structural networks in amnestic mild cognitive impairment: relationship to subtype. *Radiology*. 2012;265:518–27.
26. Lo C-Y, Wang P-N, Chou K-H, Wang J, He Y, Lin C-P. Diffusion tensor tractography reveals abnormal topological organization in structural cortical networks in Alzheimer's disease. *The Journal of neuroscience*. 2010;30:16876–85.
27. Reijmer YD, Leemans A, Brundel M, Kappelle LJ, Biessels GJ. Disruption of the cerebral white matter network is related to slowing of information processing speed in patients with type 2 diabetes. *Diabetes*. 2013;62:2112–5.
28. Reijmer YD, Leemans A, Caeyenberghs K, Heringa SM, Koek HL, Biessels GJ. Disruption of cerebral networks and cognitive impairment in Alzheimer disease. *Neurology*. 2013;80:1370–7.
29. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Archives of neurology*. 1999;56:303–8.
30. 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;34:939–44.
31. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology*. 1993;43:2412–4.
32. Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. *Journal of the American Geriatrics Society*. 1992;40:922–35.
33. Jones DK, Leemans A. Diffusion tensor imaging. *Methods in molecular biology*. 2011;711:127–44.

34. Leemans A, Jones DK. The B-matrix must be rotated when correcting for subject motion in DTI data. *Magnetic Resonance in Medicine*. 2009;61:1336–49.
35. Leemans A, Jeurissen B, Sijbers J, Jones DJ. ExploreDTI: a graphical toolbox for processing, analyzing, and visualizing diffusion MR data. *International Society for Magnetic Resonance in Medicine* - 17th Scientific Meeting in Honolulu, Hawaii. 2009. p. 3537.
36. Vos SB, Jones DK, Jeurissen B, Viergever MA, Leemans A. The influence of complex white matter architecture on the mean diffusivity in diffusion tensor MRI of the human brain. *NeuroImage*. 2012;59:2208–16.
37. Basser PJ, Pajevic S, Pierpaoli C, Duda J, Aldroubi A. In vivo fiber tractography using DT-MRI data. *Magnetic resonance in medicine*. 2000;44:625–32.
38. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage*. 2002;15:273–89.
39. Rubinov M, Sporns O. Complex network measures of brain connectivity: uses and interpretations. *NeuroImage*. 2010;52:1059–69.
40. Rubinov M, Sporns O. Weight-conserving characterization of complex functional brain networks. *NeuroImage*. 2011;56:2068–79.
41. Watts DJ, Strogatz SH. Collective dynamics of "small-world" networks. *Nature*. 1998;393:440–2.
42. Gregoire SM, Chaudhary UJ, Brown MM, Yousry TA, Kallis C, Jäger HR, Werring DJ. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. *Neurology*. 2009;73:1759–66.
43. Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjogren M, Wallin A, Ader H, Leys D, Pantoni L, Pasquier F, Erkinjuntti T, Scheltens P. A New Rating Scale for Age-Related White Matter Changes Applicable to MRI and CT. *Stroke*. 2001;32:1318–1322.
44. Scheltens P, Launer LJ, Barkhof F, Weinstein HC, van Gool WA. Visual assessment of medial temporal lobe atrophy on magnetic resonance imaging: interobserver reliability. *Journal of neurology*. 1995;242:557–60.
45. Corrigan JD, Hinkeldey NS. Relationships between parts A and B of the Trail Making Test. *Journal of clinical psychology*. 1987;43:402–9.
46. Stroop J. Studies of interference in serial verbal reactions. *Journal of Experimental Psychology*. 1935;18:643–662.

47. Van der Elst W, van Boxtel MPJ, van Breukelen GJP, 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*. 2005;11:290–302.
48. Van der Elst W, van Boxtel MPJ, van Breukelen GJP, 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.
49. Li Y, Liu Y, Li J, Qin W, Li K, Yu C, Jiang T. Brain anatomical network and intelligence. *PLoS computational biology*. 2009;5:e1000395.
50. Gong G, Rosa-Neto P, Carbonell F, Chen ZJ, He Y, Evans AC. Age- and gender-related differences in the cortical anatomical network. *The Journal of neuroscience*. 2009;29:15684–93.
51. Yao Z, Zhang Y, Lin L, Zhou Y, Xu C, Jiang T. Abnormal cortical networks in mild cognitive impairment and Alzheimer's disease. *PLoS computational biology*. 2010;6:e1001006.
52. He Y, Chen Z, Evans A. Structural insights into aberrant topological patterns of large-scale cortical networks in Alzheimer's disease. *The Journal of neuroscience*. 2008;28:4756–66.
53. Stam CJ, Jones BF, Nolte G, Breakspear M, Scheltens P. Small-world networks and functional connectivity in Alzheimer's disease. *Cerebral cortex*. 2007;17:92–9.
54. De Haan W, Pijnenburg YAL, Strijers RLM, van der Made Y, van der Flier WM, Scheltens P, Stam CJ. Functional neural network analysis in frontotemporal dementia and Alzheimer's disease using EEG and graph theory. *BMC neuroscience*. 2009;10:101.
55. Stam CJ, de Haan W, Daffertshofer A, Jones BF, Manshanden I, van Cappellen van Walsum AM, Montez T, Verbunt JPA, de Munck JC, van Dijk BW, Berendse HW, Scheltens P. Graph theoretical analysis of magnetoencephalographic functional connectivity in Alzheimer's disease. *Brain*. 2009;132:213–24.
56. Supekar K, Menon V, Rubin D, Musen M, Greicius MD. Network analysis of intrinsic functional brain connectivity in Alzheimer's disease. *PLoS computational biology*. 2008;4:e1000100.
57. Sanz-Arigita EJ, Schoonheim MM, Damoiseaux JS, Rombouts SARB, Maris E, Barkhof F, Scheltens P, Stam CJ. Loss of "small-world" networks in Alzheimer's disease: graph analysis of FMRI resting-state functional connectivity. *PloS one*. 2010;5:e13788.

58. Zhao X, Liu Y, Wang X, Liu B, Xi Q, Guo Q, Jiang H, Jiang T, Wang P. Disrupted small-world brain networks in moderate Alzheimer's disease: a resting-state FMRI study. *PloS one*. 2012;7:e33540.
59. Seo EH, Lee DY, Lee J-M, Park J-S, Sohn BK, Lee DS, Choe YM, Woo JI. Whole-brain Functional Networks in Cognitively Normal, Mild Cognitive Impairment, and Alzheimer's Disease. *PloS one*. 2013;8:e53922.
60. Xie T, He Y. Mapping the Alzheimer's brain with connectomics. *Frontiers in psychiatry*. 2012;2:77.
61. Dierksen GA, Skehan ME, Khan MA, Jeng J, Nandigam RNK, Becker JA, Kumar A, Neal KL, Betensky RA, Frosch MP, Rosand J, Johnson KA, Viswanathan A, Salat DH, Greenberg SM. Spatial relation between microbleeds and amyloid deposits in amyloid angiopathy. *Annals of neurology*. 2010;68:545–8.
62. Goos JDC, Teunissen CE, Veerhuis R, Verwey NA, Barkhof F, Blankenstein MA, Scheltens P, van der Flier WM. Microbleeds relate to altered amyloid-β metabolism in Alzheimer's disease. *Neurobiology of aging*. 2012;33:1011.e1–9.
63. Bosch B, Arenaza-Urquijo EM, Rami L, Sala-Llonch R, Junqué C, Solé-Padullés C, Peña-Gómez C, Bargalló N, Molinuevo JL, Bartrés-Faz D. Multiple DTI index analysis in normal aging, amnestic MCI and AD. Relationship with neuropsychological performance. *Neurobiology of aging*. 2012;33:61–74.
64. Jeurissen B, Leemans A, Jones DK, Tournier J-D, Sijbers J. Probabilistic fiber tracking using the residual bootstrap with constrained spherical deconvolution. *Human brain mapping*. 2011;32:461–79.
65. Bastiani M, Shah NJ, Goebel R, Roebroeck A. Human cortical connectome reconstruction from diffusion weighted MRI: the effect of tractography algorithm. *NeuroImage*. 2012;62:1732–49.

CHAPTER 7

HIGH PREVALENCE OF CEREBRAL MICROBLEEDS AT 7 TESLA MRI IN PATIENTS WITH EARLY ALZHEIMER'S DISEASE

M Brundel, SM Heringa, J de Bresser, HL Koek, JJM Zwanenburg, LJ Kappelle, PR Luijten, GJ Biessels

Published in: Journal of Alzheimer's Disease 2012;31(2):259-63.

ABSTRACT

The prevalence of microbleeds on MRI in patients with Alzheimer's disease (AD) is lower than that of its presumed pathological correlate, cerebral amyloid angiopathy. We examined 18 patients with early AD or mild cognitive impairment (MCI) and 18 non-demented controls with ultra-high field strength 7 Tesla MRI, to assess if the actual prevalence of microbleeds could be higher than is currently reported. One or more microbleeds were visualized in 78% of the MCI/AD patients and in 44% of the controls (p=0.04). 7 Tesla MRI shows that presence of microbleeds may be the rule, rather than exception in patients with MCI/AD.

INTRODUCTION

Cerebral microbleeds are small foci of signal attenuation on T2*-weighted gradient echo magnetic resonance imaging (MRI), due to iron deposits thought to reflect chronic hemosiderin deposits, that are associated with cerebrovascular disease and dementia.[1] Recently, microbleeds have attracted attention in amyloid-β immunization therapy trials in Alzheimer's disease (AD) because presence of multiple microbleeds prior to therapy is considered to reflect an increased risk of treatment-related complications.[2]

Cerebral amyloid angiopathy (CAA) is presumed to be the main neuropathological correlate of microbleeds in AD. However, there is a marked discrepancy between the prevalence of CAA in AD in autopsy studies (82-98%) and that of microbleeds on MRI (16-32%).[2,3] The number of microbleeds detected on MRI is highly dependent of scan protocol and field strength. Higher field strength provides higher spatial resolution, improves signal-to-noise ratio and shows a higher susceptibility effect. Previous studies at 7 Tesla MRI showed that even small microbleeds were visible and that the number of detected microbleeds was three times higher than at 1.5 Tesla MRI.[4,5]

The present study addressed the question whether the actual prevalence of microbleeds is much higher than previously reported, by examining patients with AD or pre-AD stages with ultra-high field 7 Tesla MRI.

METHODS

Study population

From a consecutive series of patients referred to the memory clinic of our center we included all patients who met the following criteria: diagnosis early AD or mild cognitive impairment (MCI; considered to represent a transition state between normal aging and dementia); no contra-indications for 7 Tesla MRI; Mini-Mental State Examination (MMSE)[6] score ≥20; Clinical Dementia Rating (CDR)[7] ≤1; signed informed consent. Of 19 eligible patients one was excluded because of severe motion artifacts on the MRI. This left 18 patients (with MCI (n=9), possible AD (n=2) or probable AD (n=7)). Diagnoses were established at a multidisciplinary meeting, without consideration of the 7 Tesla

MRI scan, according to internationally accepted criteria and were verified by two investigators (MB and HLK).[8,9] All participants underwent a standardized evaluation, including medical history, physical and neurological examination, laboratory testing, 3 Tesla MRI and 7 Tesla MRI.

Eighteen age-matched functionally independent non-demented controls with a MMSE score of ≥28, recruited through general practitioners in the region, served as a reference group.

The study was approved by the local medical ethics committee.

MRI scanning protocol

Scans were acquired on a 7 Tesla MR system (Philips Healthcare, Cleveland, OH, USA) with a volume transmit and 16-channel receive head coil (Nova Medical, Wilmington, MA). Dual-echo 3D T2*-weighted images (repetition time (TR)/first echo time (TE)/second TE = 20/6.9/15.8 ms, non-interpolated resolution 0.5x0.5x0.7 mm^3) were acquired. Minimum intensity projection (MinIP) processing was performed to enhance the hypointense microbleeds and reduce the large number of slices (slice thickness/overlap = 4/2 mm).

On the same day, participants also underwent 3 Tesla MRI (Philips Medical Systems, Best, the Netherlands), including a T2*-weighted sequence (TR/TE = 1653/20 ms, non-interpolated resolution 0.99x0.99x3.00 mm^3), to provide a frame of reference.

Microbleed rating

Microbleeds were rated visually by two observers, blinded to clinical information, according to the MARS criteria.[10] On 7 Tesla MRI, no lower size limit was applied and lesions visible on the first echo image had to be larger on the second echo image (so-called blooming), to be classified as microbleeds. Mimics like symmetric calcifications in the basal ganglia and choroid plexus were disregarded. Only definite microbleeds were included in analyses.

For seven randomly selected lesions identified as microbleeds on 7 Tesla MRI, the phase images were examined to verify that the lesions were indeed due to iron deposition, rather than calcifications. All lesions appeared to be paramagnetic, indicating increased concentration of paramagnetic iron.

Ratings of the two observers (MB and SMH) were compared and in case of

discordance they performed a consensus rating. Remaining disagreements were resolved by a third observer (JB) (this was the case in seven microbleeds in six patients). The inter-rater agreement of the observers for number of microbleeds at 7 Tesla MRI was excellent (ICC=0.93).

White matter hyperintensities (WMH, using the Age-Related White Matter Changes (ARWMC) rating scale[11]) and lacunes (defined as lesions <1.5 cm in diameter, with a hypointense core on T1 and FLAIR images and with an appearance unlike a perivascular space) were scored on 3 Tesla MRI.

Statistical analysis

Between-group differences were analyzed with analysis of variance for continuous variables, Mann-Whitney U tests for non-parametric data and $\chi 2$ tests for proportions. Inter-rater agreement on the number of rated microbleeds was calculated as Intraclass Correlation Coefficient (ICC). The relationship between the number of microbleeds and age, MMSE and WMH were measured with Spearman's rank correlation coefficients.

RESULTS

Participant characteristics are shown in Table 1. The groups were similar in age, gender, vascular risk factor profile and medication use ($p>0.05$). As expected, MMSE score differed significantly between groups (MCI/AD patients median(range): 26(20-29), controls: 30(28-30), $p<0.01$).

On 7 Tesla MRI, microbleed prevalence differed significantly between patients and controls: 14 (78%) of the MCI/AD patients and eight (44%) of the controls showed ≥1 microbleed ($p=0.040$) (Table 2, Figure). The proportion of subjects with microbleeds was similar in those with AD and those with MCI (seven out of nine each). MCI/AD patients had a higher total microbleed number (maximum of 80 in a single subject) than controls (maximum of five, $p=0.065$). The proportion of participants with deep/infratentorial microbleeds was also higher in patients with MCI/AD (56%) than in controls (17%) ($p=0.015$), however, the proportion of patients with strictly deep/infratentorial or strictly lobar microbleeds did not differ between MCI/AD patients and controls (strictly deep/infratentorial microbleeds: 17% and 6%; strictly lobar

Table 1. Participant characteristics

	MCI/AD patients	Controls	p-value
Participants (n)	18	18	
Age (years)	74.3±8.6	72.0±4.5	0.334
Male sex	8 (44)	12 (67)	0.180
Hypertension [a]	9 (50)	9 (50)	1.000
Hypercholesterolemia [a]	8 (44)	8 (44)	1.000
Diabetes mellitus [a]	3 (17)	3 (17)	1.000
Current smoking	3 (17)	2 (11)	0.630
Antithrombotic use	9 (50)	6 (33)	0.298
Systolic blood pressure (mmHg)	148±22	154±26	0.467
Diastolic blood pressure (mmHg)	80±12	82±9	0.499
MMSE score (median, range)	26 (20-29)	30 (28-30)	0.000
Lacunar infarcts [b]	6 (33)	6 (33)	1.000
White matter hyperintensities [b] (median, range)	4.25 (1.5-17.5)	4.75 (0.5-17)	0.434

Data are mean ±SD or n(%) unless otherwise specified

[a] Patients were considered having arterial hypertension, hypercholesterolemia or diabetes mellitus, if they had a known history of the disease or were receiving drug treatment for these conditions.

[b] scored at 3 Tesla MRI, for white matter hyperintensities the total ARWMC score is shown

microbleeds: 22% and 28% respectively, p>0.05). Among participants with strictly deep/infratentorial or strictly lobar microbleeds, microbleed numbers did not differ significantly between the MCI/AD patients and controls (strictly deep/infratentorial microbleeds (median(range): 1 (1-3) / 1 (1-1), p=0.564; strictly lobar microbleeds: 1 (1-2) / 1 (1-4), p=0.467).

We found no statistically significant relationship between number of microbleeds at 7 Tesla MRI and age (correlation coefficient MCI/AD patients: 0.308, p=0.214; controls 0.224, p=0.372); MMSE (MCI/AD patients: 0.390, p=0.110; controls: -0.226, p=0.368); or WMH (MCI/AD patients: 0.399, p=0.101; controls: 0.027, p=0.916).

By comparison to 7 Tesla MRI, on 3 Tesla MRI, microbleeds were detected in only six (33%) of the MCI/AD patients and three (17%) of the control participants (p=0.248) (Table 2). The highest number of microbleeds in a single subject was ten. One hundred thirteen microbleeds identified at 7 Tesla MRI, were not identified at 3 Tesla MRI. Four microbleeds identified at 3 Tesla MRI, were not identified at 7 Tesla MRI. Two of these were located in the basal ganglia, and were obscured by low signal intensity in this area at 7 Tesla MRI, probably due to local iron depositions; two other microbleeds (both lobar) were not identified at 7 Tesla MRI due to motion artifacts.

DISCUSSION

This study demonstrates that with optimized MR scan techniques at ultra-high field strength, the majority of MCI/AD patients exhibit cerebral microbleeds. Microbleed prevalences in patients (78%) and controls (44%) are much higher than those in the literature. Previous studies in AD patients at lower field strength (1.0-2.3 Tesla), including large cohorts and a recent meta-analysis by Cordonnier et al., reported prevalences around 25%.[2] At 3 Tesla field strength, a previous study in AD patients described a slightly higher microbleed prevalence of 31%.[12] For non-demented individuals around the age of 70 (like our controls) reported prevalences vary between 5-28% at lower field strength (1.0-1.5 Tesla)[12,13] and are 19% at 3 Tesla MRI.[12]

Observations from an increasing number of studies at regular field strength point to the potential clinical relevance of microbleeds. Prevalence,

Table 2. Microbleed prevalence and number

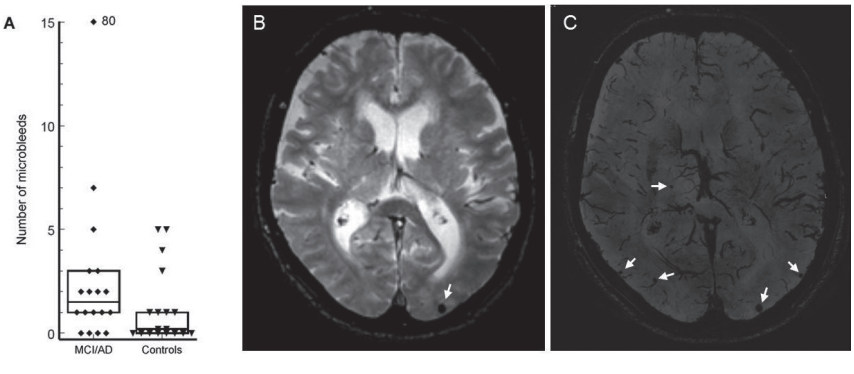
	Any microbleeds		
	MCI/AD	Controls	p-value
7 Tesla MRI			
Prevalence (%)	14 (78)	8 (44)	0.040*
Number in total group	1.5 (0-80)	0 (0-5)	0.065
Number in MB+ group [a]	2 (1-80)	2 (1-5)	0.887
3 Tesla MRI			
Prevalence (%)	6 (33)	3 (17)	0.248
Number in total group	0 (0-10)	0 (0-1)	0.151
Number in MB+ group [a]	2 (1-10)	1 (1-1)	0.039*

Data are presented as n(%) or median (range).

[a] MB+ group: microbleed numbers only among participants with microbleeds

* $p<0.05$

Figure. Microbleeds at 3 Tesla and 7 Tesla MRI



Total number of microbleeds at 7 Tesla MRI in patients with MCI/AD and controls (box represents median with interquartile range) (A) and an example of a single slice T2*-weighted MR image at 3 Tesla MRI (B) and 7 Tesla MRI (second echo) (B), showing four extra microbleeds at 7 Tesla MRI (arrows).

Deep/infratentorial microbleeds			Lobar microbleeds		
MCI/AD	Controls	p-value	MCI/AD	Controls	p-value
10 (56)	3 (17)	0.015*	11 (61)	7 (39)	0.182
1 (0-5)	0 (0-3)	0.026*	1 (0-75)	0 (0-4)	0.321
1 (1-5)	2 (1-3)	0.562	1 (1-75)	2 (1-4)	0.485
5 (28)	2 (11)	0.206	5 (28)	1 (6)	0.074
0 (0-1)	0 (0-1)	0.213	0 (0-9)	0 (0-1)	0.071
1 (1-1)	1 (1-1)	1.000	1 (1-9)	1 (1-1)	0.488

number and localization of cerebral microbleeds have been suggested to convey important prognostic and etiological information. Microbleeds have been linked to worse cognitive functioning[14] and an increased risk of cognitive decline and dementia in patients with MCI.[15,16] Microbleeds are also starting to influence treatment decisions.[2] Recently, presence of microbleeds has been introduced as an exclusion criterion in clinical trials of amyloid β immunization therapy. The rationale behind this is that multiple microbleeds are considered to be a marker of advanced CAA, which has been linked to immunization-related complications, such as vasogenic edema.[2,17] Moreover, because people with microbleeds, with or without a previous clinically manifest stroke, appear to be at increased risk of future hemorrhagic stroke, the question has been raised if presence of microbleeds should influence the prescription of antithrombotic agents.[1] It is still unknown, however, if these agents increase the risk of future hemorrhages in patients with microbleeds.[18]

Importantly, current knowledge is based on studies using 1.0-3.0 Tesla MRI. The present exploratory study indicates that these studies underestimated the actual prevalence of microbleeds, and that microbleeds might be considered almost generic to AD. This would also fit with the high prevalence of CAA in

AD in neuropathological studies.[3] Furthermore, based on studies at regular field strength, it has been suggested that the location of cerebral microbleeds is related to etiology, with deep and infratentorial microbleeds related to hypertensive vasculopathy and lobar microbleeds to CAA.[19,20] We did not find differences in the proportions of patients with strictly deep/infratentorial or lobar microbleeds. Possibly, the relation of microbleed location to specific etiologies may not be as clear as has previously been suggested. It is also possible that the microbleeds that are only visible at higher field strength reflect other vascular pathologies than those that are also observed at lower field strength. Larger prospective studies, also at high field strength, will be needed to unravel the true prognostic and etiologic relevance of microbleeds in AD.

A limitation of our study is the modest sample size. This may also explain the absence of statistically significant correlations between the presence of microbleeds and age. Moreover, the stringent contra-indications for 7 Tesla MRI might have caused selection bias. However, the prevalence of microbleeds at 3 Tesla MRI in the present study is well in line with previous reports, indicating that we recruited representative participants. Finally, we combined data from patients with early AD and MCI. Although MCI is a pre-AD stage in a proportion of patients, MCI may have different etiologies and cognition can even return to normal.[8] We have nevertheless chosen to include patients with MCI because the earliest stages of AD pathology can be observed among them.

REFERENCES

1. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, Launer LJ, Van Buchem MA, Breteler MM. Cerebral microbleeds: a guide to detection and interpretation. *Lancet neurology*. 2009;8:165–74.
2. Cordonnier C, van der Flier WM. Brain microbleeds and Alzheimer's disease: innocent observation or key player? *Brain*. 2011;134:335–44.
3. Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. *Journal of neural transmission*. 2002;109:813–36.
4. Conijn MMA, Geerlings MI, Biessels GJ, Takahara T, Witkamp TD, Zwanenburg JJM, Luijten PR, Hendrikse J. Cerebral microbleeds on MR imaging: comparison between 1.5 and 7T. *American journal of neuroradiology*. 2011;32:1043–9.
5. Theysohn JM, Kraff O, Maderwald S, Barth M, Ladd SC, Forsting M, Ladd ME, Gizewski ER. 7 tesla MRI of microbleeds and white matter lesions as seen in vascular dementia. *Journal of magnetic resonance imaging*. 2011;33:782–91.
6. 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;12:189–98.
7. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. *The British journal of psychiatry*. 1982;140:566–72.
8. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Archives of neurology*. 1999;56:303–8.
9. 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;34:939–44.
10. Gregoire SM, Chaudhary UJ, Brown MM, Yousry TA, Kallis C, Jäger HR, Werring DJ. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. *Neurology*. 2009;73:1759–66.
11. Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjogren M, Wallin A, Ader H, Leys D, Pantoni L, Pasquier F, Erkinjuntti T, Scheltens P. A New Rating Scale for Age-Related White Matter Changes Applicable to MRI and CT. *Stroke*. 2001;32:1318–1322.

12. Yates PA, Sirisriro R, Villemagne VL, Farquharson S, Masters CL, Rowe CC. Cerebral microhemorrhage and brain β-amyloid in aging and Alzheimer disease. *Neurology*. 2011;77:48–54.
13. Jeerakathil T, Wolf PA, Beiser A, Hald JK, Au R, Kase CS, Massaro JM, DeCarli C. Cerebral microbleeds: prevalence and associations with cardiovascular risk factors in the Framingham Study. *Stroke*. 2004;35:1831–5.
14. Poels MMF, Ikram MA, van der Lugt A, Hofman A, Krestin GP, Breteler MMB, Vernooij MW. Incidence of cerebral microbleeds in the general population: the Rotterdam Scan Study. *Stroke*. 2011;42:656–61.
15. Staekenborg SS, Koedam ELGE, Henneman WJP, Stokman P, Barkhof F, Scheltens P, van der Flier WM. Progression of mild cognitive impairment to dementia: contribution of cerebrovascular disease compared with medial temporal lobe atrophy. *Stroke*. 2009;40:1269–74.
16. Kirsch W, McAuley G, Holshouser B, Petersen F, Ayaz M, Vinters H V, Dickson C, Haacke EM, Britt W, Larseng J, Kim I, Mueller C, Schrag M, Kido D. Serial susceptibility weighted MRI measures brain iron and microbleeds in dementia. *Journal of Alzheimer's disease*. 2009;17:599–609.
17. Sperling RA, Jack CR, Black SE, Frosch MP, Greenberg SM, Hyman BT, Scheltens P, Carrillo MC, Thies W, Bednar MM, Black RS, Brashear HR, Grundman M, Siemers ER, Feldman HH, Schindler RJ. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials : Recommendations from the Alzheimer ' s Association Research Roundtable Workgroup. *Alzheimer's & Dementia*. 2011;7:367–385.
18. Vernooij MW, Haag MDM, van der Lugt A, Hofman A, Krestin GP, Stricker BH, Breteler MMB. Use of antithrombotic drugs and the presence of cerebral microbleeds: the Rotterdam Scan Study. *Archives of neurology*. 2009;66:714–20.
19. Cullen KM, Kócsi Z, Stone J. Microvascular pathology in the aging human brain: evidence that senile plaques are sites of microhaemorrhages. *Neurobiology of aging*. 2006;27:1786–96.
20. Fazekas F, Kleinert R, Roob G, Kleinert G, Kapeller P, Schmidt R, Hartung HP. 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. *American journal of neuroradiology*. 1999;20:637–42.

CHAPTER 8

CEREBRAL CORTICAL MICROINFARCTS AT 7 TESLA MRI IN PATIENTS WITH EARLY ALZHEIMER'S DISEASE

SJ van Veluw, SM Heringa, HJ Kuijf, HL Koek, PR Luijten, GJ Biessels;
on behalf of the Utrecht Vascular Cognitive Impairment study group

Published in: Journal of Alzheimer's Disease 2014 (in press).

ABSTRACT

Cerebral microinfarcts (CMIs) are a common finding in neuropathological studies of aging and dementia. Recently, it has become possible to detect CMIs in vivo. We studied CMI occurrence in 29 patients with mild cognitive impairment or early Alzheimer's disease (AD) and 22 non-demented individuals on 7 Tesla MRI. CMI occurrence in patients (55%) and controls (45%) was not significantly different. In patients, CMI number tended to be related to microbleed number (p=0.07). This first in vivo study of CMIs in early AD does not confirm findings from autopsy studies. Further studies are needed to clarify the role of CMIs in AD.

INTRODUCTION

Cerebral microinfarcts (CMIs) are commonly observed in brain aging, cerebrovascular disease, and dementia in autopsy studies.[1,2] A systematic review of these studies reported frequencies of 43% in patients with Alzheimer's disease (AD; n=409), and 24% in non-demented older adults (n=1229).[1] CMIs occur widespread throughout the brain, and lesion counts have been estimated of up to a thousand per patient.[3] CMIs are associated with worse cognitive functioning, also independent of Alzheimer pathology.[4,5] Due to their small size, ranging from 50 μm to a few mm, CMIs are hard to detect on conventional MRI. Recently, it has been demonstrated that cortical CMIs can be visualized using ultra-high field 7 Tesla MRI.[6] This finding enables, for the first time, to study CMIs in vivo. In the current study, we compared the occurrence of cortical CMIs between patients in an early stage of AD or mild cognitive impairment (MCI) and controls. Furthermore, we explored the relation of cortical CMIs with other imaging markers of small vessel disease.

METHODS

Study population

Thirty-two patients were recruited via a memory clinic of the University Medical Center Utrecht (UMCU) in the period February 2011–November 2012. Inclusion criteria were: diagnosis of early possible or probable AD (according to the NINCDS-ADRDA criteria[7]), or amnestic MCI (considered to represent a transitional stage between normal aging and AD[8]); no contraindications for 7 Tesla MRI (e.g. metal objects in or on the body, claustrophobia); Mini-Mental State Examination (MMSE) ≥20; clinical dementia rating scale (CDR) ≤1. Clinical diagnoses were established at a multidisciplinary meeting.

A reference group of 24 functionally independent, non-demented older participants was recruited via their general practitioners during the same period. Inclusion criteria were: no known history of neurological disease or psychiatric disorder; no contraindications for 7 Tesla MRI; MMSE >25.

Three patients with MCI or early AD and two control participants were excluded because of severe motion artifacts on their MR images, leaving

29 patients and 22 control participants included in the current study. All participants underwent a standard evaluation, including medical history, physical, neurological, and neuropsychological examination, 7 Tesla and 3 Tesla MRI on the same day. The study was approved by the medical ethics committee of the UMCU and all participants gave written informed consent.

MRI scanning protocol

7 Tesla scans were acquired on a whole-body MR system (Philips Healthcare, Cleveland, OH, USA) with a volume transmit and 16 or 32-channel receive head coil (Nova Medical, Wilmington, MA, USA). The standardized protocol included 3D fluid attenuated inversion recovery (FLAIR) (TR/TE/TI=8000/300/2325 ms; voxel size 0.8x0.8x0.8 mm^3), 3D T2 weighted (TR/TE=3158/301 ms; voxel size 0.7x0.7x0.7 mm^3), 3D T1 weighted (TR/TE/TI=4.8/2.2/1240 ms; voxel size 1.0x1.0x1.0 mm^3), and 3D dual-echo gradient echo (TR/TE1/TE2= 20/6.9/15.8 ms; voxel size 0.5x0.5x0.7 mm^3) images.

The standardized 3 Tesla MRI protocol (Intera, Philips, Best, The Netherlands) included FLAIR (TR/TE/TI=11000/125/2800 ms; voxel size 1.0x1.3x3.0 mm^3) and 3D T1 weighted (TR/TE/TI=7.9/4.5/955 ms; voxel size 1.0x1.0x1.0 mm^3) images.

MRI rating of CMIs

Cortical CMIs were scored on 7 Tesla FLAIR, T2, and T1 weighted images. All ratings were performed blinded to clinical information. For visual rating we used a previously established operational definition of cortical CMIs[6]: the lesion had to be hyperintense on both FLAIR and T2 weighted images, and hypointense on the T1 weighted image. It had to be restricted to the cortex, distinct from perivascular spaces, and ≤3 mm in length.

First, all scans were evaluated according to these criteria by an experienced rater (intra-rater reliability: intraclass correlation coefficient (ICC): 0.98; Dice's similarity coefficient (DSC): 0.70). This first visual rating yielded 40 cortical CMIs. Next, the visual rating was complemented by a semi-automated detection method. This method was designed to automatically indicate cortical scan abnormalities possibly representing CMIs, and was found to increase the number of detected lesions over visual rating alone.[9] In short, the cortex was

segmented on the 3 Tesla T1 weighted image using FreeSurfer. With the use of elastix, the 3 Tesla T1 weighted sequence, the cortex segmentation, the 7 Tesla T1 and T2 weighted sequences were registered to the 7 Tesla FLAIR sequence. Next, locations were marked that had a local maximum in signal intensity on both FLAIR and T2, were distinct from perivascular spaces, fulfilled the size-requirements, and were close and connected to the pial surface.

Two independent raters evaluated the locations that were indicated by the semi-automated method, according to our CMI criteria. In case of disagreement a consensus meeting was held. By applying the semi-automated method, 37 additional CMIs were found. CMIs that were not detected by the semi-automated method, but had been rated in the first visual rating (n=12), were discussed in the consensus meeting as well, resulting in the rejection of four of these CMIs.

MRI rating of other markers of small vessel disease

All MRI ratings were performed by two human raters, blinded to clinical information. In case of disagreement a consensus meeting was held. Cerebral microbleeds (CMBs) were rated on 7 Tesla dual-echo gradient echo images with a semi-automated detection method, followed by visual censoring by two raters.[10] Other, larger, cortical infarcts (>3 mm) were assessed on 7 Tesla FLAIR images. Lacunar infarcts, white matter hyperintensities (using the age-related white matter changes (ARWMC) scale),[11] and medial temporal lobe atrophy (MTA)[12] were assessed on 3 Tesla FLAIR images. Intracranial volume was calculated automatically using the 3 Tesla T1 weighted image in FreeSurfer.

Statistical analysis

Inter-rater reliability for cortical CMI rating was measured using the ICC and DSC. Between-group differences were analyzed with Mann-Whitney U tests for non-parametric data, and chi-square tests for proportions. Correlations were assessed using Spearman's rank correlation coefficient for non-parametric data.

An explorative analysis on the relation between CMIs and vascular risk factors, cognition, and other MRI markers was performed. For the cognitive domain memory, within the patient group, the raw test scores of the Rey auditory verbal learning test and visual association test were standardized into z-scores and averaged.

Table 1. Participant characteristics

	Patients with MCI or early AD n=29	Control participants n=22	P-value
Age (years)	74.8 ± 8.4	68.8 ± 3.3	0.003*
Male gender	12 (41%)	9 (41%)	0.973
Hypertension[a]	14 (48%)	11 (50%)	0.903
Diabetes mellitus[a]	2 (7%)	5 (23%)	0.104
Systolic blood pressure (mmHg)	155 ± 19	142 ± 14	0.009*
Diastolic blood pressure (mmHg)	83 ± 8	81 ± 5	0.358
MMSE score	26 [20-29]	29 [26-30]	0.000*
Intracranial volume (ml)	1469 ± 145	1434 ± 211	0.418
MTA score	2 [0-3]	0 [0-2]	0.000*

Data are presented as mean ± SD, n (%), or median [range].

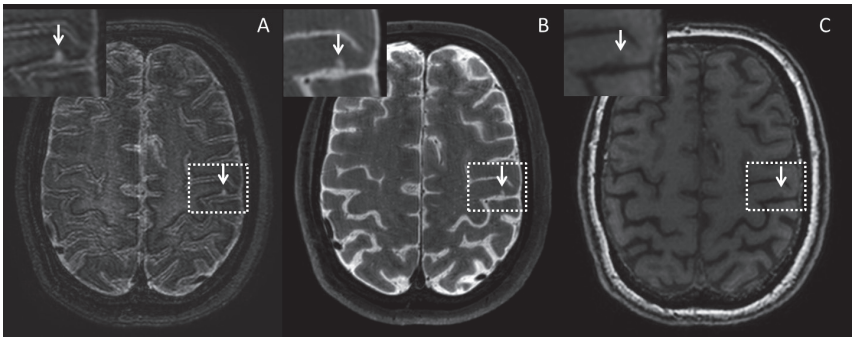
[a] Participants were considered as having arterial hypertension or diabetes mellitus in case of known history or drug treatment.

* Statistically significant difference between groups.

MMSE: mini-mental state examination; MTA: medial temporal lobe atrophy.

Figure 1. A cortical microinfarct in a 62-year old male with possible AD

The cortical microinfarct appears hyperintense on FLAIR (A), hyperintense on T2 (B), and hypointense on T1 (C).



RESULTS

Subject characteristics are presented in Table 1. Patients with MCI (n=17) or early AD (n=12) (mean age 74.8±8.4 years) were slightly older than control subjects (n=22; mean age 68.8±3.3 years). As expected, patients had significant lower MMSE and higher MTA scores than controls. The groups were similar in gender distribution, vascular risk factor profile, and mean intracranial volume. A total number of 73 CMIs were found (Figure 1). Inter-rater agreement on the evaluation of locations marked by the semi-automated method was moderate to good (ICC: 0.76; DSC: 0.46).

A total number of 40 cortical CMIs were found in 16 (55%) patients with MCI or early AD, whereas a total number of 33 cortical CMIs were found in 10 (45%) control participants. CMI occurrence was not significantly different between the two groups ($p>0.05$; Table 2). Within the patient group, CMI occurrence did not differ between patients with MCI or early AD. In both groups, CMIs were uniformly distributed throughout the cortex with no predilection for specific brain areas (Supplementary Figure 1).

Explorative analyses on clinical correlates of CMIs showed that hypertension tended to be related to higher numbers of CMIs in the control group ($p=0.06$), but not in the patient group. CMIs were not related to MTA or cognition in either group. Within the patient group, CMI number tended to be related to CMB number ($p=0.07$). Moreover, CMIs were related to other, larger, cortical infarcts in both groups, but not to other subcortical vascular lesions. More details are provided in Supplementary Table 1.

DISCUSSION

This is the first MRI study that assessed the occurrence and number of CMIs in patients with MCI or early AD. CMI numbers were not significantly increased in patients relative to controls. They did appear to be related to CMB numbers and other, larger, cortical infarcts.

So far, CMIs have only been studied in post-mortem brain tissue. Based on results from such neuropathological studies, we had expected to find more CMIs in our patient group than in controls.[1] Our negative results may be explained by

Table 2. CMIs and other imaging markers of small vessel disease

		Patients with MCI or early AD (n=29)	Control participants (n=22)	P-value
Cortical cerebral microinfarcts	Occurrence	16 (55%)	10 (45%)	0.492
	Number	1 [0-11]	0 [0-16]	0.534
Cortical infarcts (>3 mm)		2 (7%)	2 (9%)	0.773
Cerebral microbleeds	Occurrence	18 (64%)	10 (48%)	0.243
	Number	1 [0-36]	0 [0-16]	0.130
White matter hyperintensities		5 [0-17.5]	3 [0.5-17]	**0.011***
Lacunar infarcts		12 (41%)	7 (32%)	0.484

Data are presented as n (%), or median [range]. *Statistically significant difference between groups.

differences in patient characteristics. Patients with AD in autopsy studies are more likely to show end-stage pathology of their disease. In this study we have included patients in an early stage of the disease, with relatively mild impaired cognition, who may develop more CMIs over time. Furthermore, the patients and controls had similar vascular risk factors, which may be part of the etiology of CMIs. Nevertheless, these apparent discordant results strongly emphasize the importance of in vivo verification of post-mortem studies. In vivo MRI allows for (longitudinal) studies that investigate the development of CMIs, their clinical correlates, and their distribution across the brain (i.e. exploring possible predilection areas such as the watershed regions). Another advantage of MRI is whole-brain coverage, whereas standard neuropathological evaluation can capture only a limited number of brain tissue samples. On the other hand, with MRI, only the tip of the iceberg in total CMI load is within the range of detection, whereas microscopic examination of CMIs gains higher resolution.

Despite our negative findings, previous neuropathological literature strongly support the clinical relevance of CMIs. Due to their widespread

appearance, CMIs could disrupt cortical areas and may therefore contribute to cognitive decline and dementia.[2,3] Other studies have indeed shown that CMIs are related to dementia, also independently of Alzheimer pathology.[4,5]

A limitation of this study is the relatively small sample size, which limits the power for statistical analyses. Due to the strict safety regulations for 7 Tesla MRI, it is a challenge to include large numbers of participants. Selection bias could therefore be an issue. Furthermore, in cognitively impaired persons movement artifacts often occur, which can seriously hamper reliable detection of small lesions such as CMIs. Patients were older than control participants. It is unlikely that this influenced the outcome of the study, as this would have increased the contrast between the groups. Finally, cerebrospinal fluid analysis or PET data were not available to support the diagnosis of possible or probable AD.

In conclusion, contrary to our expectations, we observed no difference in CMI presence and number between patients with MCI or early AD and controls. Further MRI studies in larger groups of patients, including those with more advanced stages of AD, are needed to fully investigate CMIs and their clinical correlates in AD.

REFERENCES

1. Brundel M, de Bresser J, van Dillen JJ, Kappelle LJ, Biessels GJ. Cerebral microinfarcts: a systematic review of neuropathological studies. *Journal of cerebral blood flow and metabolism*. 2012;32:425–36.
2. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesions. *Lancet neurology*. 2012;11:272–82.
3. Westover MB, Bianchi MT, Yang C, Schneider JA, Greenberg SM. Estimating cerebral microinfarct burden from autopsy samples. *Neurology*. 2013;80:1365–9.
4. Launer LJ, Hughes TM, White LR. Microinfarcts, brain atrophy, and cognitive function: the Honolulu Asia Aging Study Autopsy Study. *Annals of neurology*. 2011;70:774–80.
5. Kalaria RN. Cerebrovascular disease and mechanisms of cognitive impairment: evidence from clinicopathological studies in humans. *Stroke*; 2012;43:2526–34.
6. Van Veluw SJ, Zwanenburg JJM, Engelen-Lee J, Spliet WGM, Hendrikse J, Luijten PR, Biessels GJ. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. *Journal of cerebral blood flow and metabolism*. 2013;33:322–9.
7. 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;34:939–44.
8. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Archives of neurology*. 1999;56:303–8.
9. Kuijf HJ, Zijlstra F, van Veluw SJ, Viergever MA, Vincken KL, Biessels GJ. Detecting cortical cerebral microinfarcts in 7.0 T MR Images. *IEEE International Symposium on Biomedical Imaging: From Nano to Macro*. 2013;982–995.
10. Kuijf HJ, de Bresser J, Geerlings MI, Conijn MMA, Viergever MA, Biessels GJ, Vincken KL. Efficient detection of cerebral microbleeds on 7.0 T MR images using the radial symmetry transform. *NeuroImage*. 2012;59:2266–73.
11. Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjogren M, Wallin A, Ader H, Leys D, Pantoni L, Pasquier F, Erkinjuntti T, Scheltens P. A New Rating Scale for Age-Related White Matter Changes Applicable to MRI and CT. *Stroke*. 2001;32:1318–1322.

12. Scheltens P, Leys D, Barkhof F, Huglo D, Weinstein HC, Vermersch P, Kuiper M, Steinling M, Wolters EC, Valk J. Atrophy of medial temporal lobes on MRI in "probable" Alzheimer's disease and normal aging: diagnostic value and neuropsychological correlates. *Journal of Neurology, Neurosurgery & Psychiatry*. 1992;55:967–972.

Supplementary Table Explorative analyses on the relation between microinfarcts and vascular risk factors, cognition, and other MRI markers

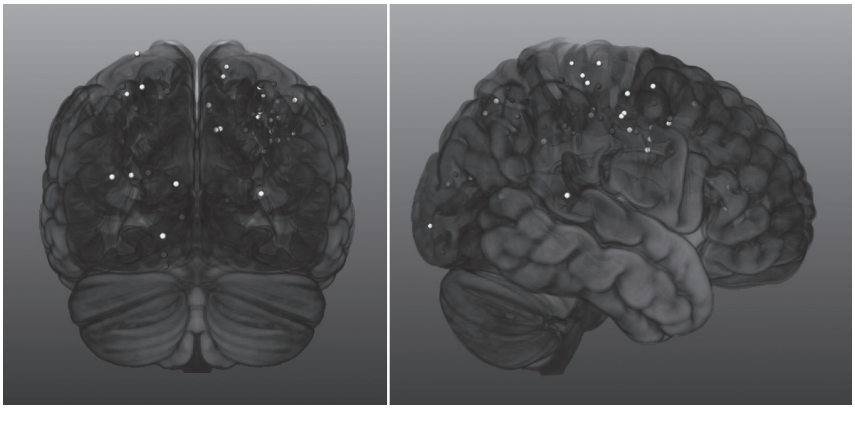
	Patients with MCI or early AD n=29	
	With CMIs n=16	Without CMIs n=13
Age (years)	73.8 ± 7.8	76.2 ± 9.3
Hypertension	6 (38%)	10 (77%)
Systolic blood pressure (mmHg)	156 ± 17	155 ± 22
MMSE score	26 [20-29]	25 [20-29]
Memory (z-score)	-0.06 ± 1.08	0.08 ± 0.93
MTA score	2 [0-3]	0 [0-2.5]
Cortical infarcts (> 3 mm)	0 [0-3]	0 [0-0]
Cerebral microbleeds	2 [0-36]	1 [0-3]
White matter hyperintensities	4 [0-17.5]	10 [2-16]
Lacunar infarcts	0 [0-5]	0 [0-9]

Data are presented as n (%), or median [range]. Columns 2, 3, 5, 6 show the clinical features of participants with and without CMIs. Columns 4 and 7 report the relation between the number of CMIs and vascular risk factors, cognition, and other MRI markers assessed by determining Spearman's rank correlation coefficient, (x) except for the relation between hypertension (yes/no) and CMI number, which was assessed with a Mann-Whitney U test for non-parametric data.

	Control participants n=22		
Correlation (R) with CMI number; p-value	With CMIs n=10	Without CMIs n=12	Correlation (R) with CMI number; p-value
-0.23; 0.229	70.0 ± 4.1	67.8 ± 2.2	0.13; 0.561
p=0.712x	7 (70%)	3 (25%)	p=0.056x
0.05; 0.785	142 ± 17	142 ± 13	0.14; 0.546
-0.08; 0.683	29.5 [27-30]	28.5 [26-30]	0.28; 0.216
-0.04; 0.830	-	-	-
0.16; 0.405	1 [0-2]	0 [0-1.5]	0.29; 0.214
0.37; 0.046	0 [0-1]	0 [0-0]	0.55; 0.008
0.35; 0.067	1 [0-4]	0 [0-3]	0.16; 0.490
0.03; 0.870	3 [0.5-17]	3 [0.5-6]	0.23; 0.297
-0.11; 0.558	0 [0-1]	0 [0-1]3 (25)	0.23; 0.307

Supplementary Table Explorative analyses on the relation between microinfarcts and vascular risk factors, cognition, and other MRI markers

All lesions are depicted that were identified as microinfarcts in patients with MCI or early AD (in white) or control participants (in grey). The temporal lobes proved difficult to assess, because of a low signal-to-noise ratio on 7 Tesla FLAIR in these areas.



PART III

EXPERIMENTAL NEUROPSYCHOLOGY

CHAPTER 9

THREE ASPECTS OF SPATIAL NAVIGATION IN HEALTHY OLDER PEOPLE AND PATIENTS WITH AMNESTIC MILD COGNITIVE IMPAIRMENT OR ALZHEIMER'S DISEASE

SM Heringa, E van den Berg, LEM Wisse, HL Koek,
LJ Kappelle, GJ Biessels, IJM van der Ham
On behalf of the Vascular Cognitive Impairment Study Group

In preparation.

ABSTRACT

Navigation is often impaired in patients with amnestic mild cognitive impairment (aMCI) or Alzheimer's disease (AD). Thirty-three patients with aMCI (73±9 years), 41 patients with AD (78±9 years), and 43 controls (74±5 years) completed the Virtual Tübingen task. After viewing a route through a virtual environment, we tested whether participants recognized scenes, remembered turns at decision points, and remembered the order of scenes. For all subtests, performance was best for controls, intermediate for patients with aMCI, and worst for patients with AD. This study shows that three important aspects of navigation are impaired in patients with aMCI or AD.

INTRODUCTION

Navigation, finding your way in well-known or new environments, is crucial for daily functioning. Wayfinding is often impaired in patients with amnestic mild cognitive impairment (aMCI) or Alzheimer's disease (AD). The result of this, getting lost and being disoriented, poses a burden on the lives of patients and their caregivers.[1] Despite the evident relevance of navigation skills, assessment of navigation is not part of standard neuropsychological examinations in general or in patients with aMCI or AD.

Spatial navigation is a complex process, which relies on several fundamental cognitive functions, such as perception, memory, attention, and decision-making.[2] Different navigational strategies can be roughly divided into two types: observer-based (egocentric) and environment-based (allocentric). In both types of strategies, landmarks are being processed and used for navigation. Allocentric navigation, however, also depends on processing of geometrical cues.[3] Accuracy of navigational performance varies largely across individuals. Individual differences, with regard to cognitive functions, strategy preferences, and flexibility in strategy use, contribute to the level of success in navigation.[2] Aging also plays an important role. Age-related decrements in basic cognitive processes, for example reduced information processing efficiency, executive functions, or accuracy of visual motion processing, lead to reduced navigation abilities.[2] Early specific navigation decrements that occur in aging typically involve allocentric processing, which is reflected in problems with forming a cognitive map through path integration, and switching to the most efficient strategy.[4,5] Navigation deficits well beyond the decrements occurring in normal aging have been reported in patients with amnestic mild cognitive impairment (e.g.[6–8]) or AD (e.g.[9–11]). These conditions are characterized clinically by cognitive disturbances, especially in the memory domain. Interestingly, navigational problems in patients with aMCI or AD have been reported to be unrelated to verbal memory performance, which implies a specific spatial deficit that is at least partially isolated from other cognitive deficiencies.[12,13] Patients with aMCI are found to function at an intermediate level for navigation performance between older controls and patients with AD.[6,13–15]

The complexity of spatial navigation is reflected in the network of brain regions that is involved in this process. Neural structures that are crucial for navigation include the hippocampus, the retrosplenial cortex, the parietal cortex, and the parahippocampal gyrus incorporating the entorhinal cortex. The hippocampus is active in processes of path integration, scene recognition, and detection of novelty, which are necessary for establishing allocentric spatial representations.[16–19] The retrosplenial cortex is activated during scene recognition,[20] the parietal cortex is involved in egocentric navigation,[16] and the parahippocampal gyrus is involved in scene perception.[21] Of these structures, the hippocampus in particular has received a lot of attention in relation to navigation. Aging-related hippocampal volume loss[22] is associated with reduced allocentric navigation performance.[23] In patients with aMCI or AD, the hippocampus is particularly damaged, which offers a neural explanation for the substantial navigation problems that occur in patients with aMCI or AD.

The exact nature of cognitive processes underlying navigational deficits in patients with aMCI or AD, however, is not yet fully known. Moreover, it is unknown whether deficits in navigation performance reflect specific problems in spatial processing, or rather are subject to non-spatial cognitive functions. Given the complexity of navigation and its dependence on multiple cognitive processes, it is of interest to assess different aspects of navigation. Therefore, we aimed to selectively assess whether three aspects of navigation, namely scene recognition, route continuation, and route ordering, were impaired in patients with aMCI and AD. We performed additional tests in order to further explore the nature of navigational deficits. Non-spatial recognition and ordering were tested to see whether any navigational deficits were specific to spatial functions. We expected that patients with aMCI or AD would perform worse than controls on three navigation aspects, and that patients with aMCI would perform at an intermediate level between healthy older controls and patients with AD. Furthermore, we expected that navigation deficits are specifically spatial, and that therefore a different performance profile is found for non-spatial control tasks. We also performed a standard neuropsychological examination on the domains memory, executive functioning, and information processing speed, to explore whether patients employ different cognitive strategies than controls.

METHODS

Participants

Thirty-three patients with aMCI (mean age 73±9 years, 39% men) and 41 patients with AD (mean age 78±9 years, 54% men; mostly at an early dementia stage), were recruited via the memory clinic at the University Medical Center Utrecht. The clinical diagnosis aMCI or AD was established at a multidisciplinary meeting. aMCI was diagnosed according to the Petersen criteria,[24] probable or possible AD was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria.[25] In addition, a control group was recruited via their general practitioners, consisting of 43 older individuals (mean age 74±5 years, 49% men), who were without known cognitive impairment (MMSE ≥ 24) and were matched for age, sex, and education. Exclusion criteria were a history of stroke in the last 2 years, a history of stroke with subsequent cognitive deterioration, schizophrenia or other psychotic disorders, major depression, alcohol abuse, brain tumor, epilepsy, or encephalitis.

The study was approved by the medical ethics committee of the University Medical Center Utrecht, the Netherlands and was conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all participants.

Task design and procedure

Navigation skills were assessed with the Virtual Tübingen task, a virtual reality environment of the town of Tübingen, Germany.[26,27] Participants were comfortably seated in front of a laptop. The task consisted of several elements: a study phase, a scene recognition test, a route continuation test and a route order test. The study phase consisted of watching a six-minute movie in which a route in a virtual environment was shown, at a set speed equal to a comfortable walking speed. After the study phase, the scene recognition was tested. The participants were shown 16 static images, half of which were stills taken from the movie and the other half was a collection of images taken from other points in the environment not visible in the movies (Figure 1). For each image, the participants were asked to indicate whether or not

Figure 1. An example of an image taken from the route
This image is taken from the starting point (reproduced from Van der Ham et al.[26]).

they had seen it before. The second test involved route continuation; for seven images taken from the route, the participants were asked to indicate whether the route continued with a left turn, straight ahead or a right turn. The last test concerned route ordering. Nine images were shown simultaneously on the screen, all taken from the route. The participants indicated the correct order of the images when walking the route from start to finish. Two points were given for each correctly ranked image, and one point for each image that was ranked one position too early or too late. The experimenter was present throughout the experiment to introduce the tests and repeat the instructions given on the screen if necessary. The tasks were presented in a fixed order to avoid unwanted training effects (e.g. stimuli presented during the continuation task could help performance on the recognition task if they were presented in this reversed order).

Furthermore, a non-spatial control task for the scene recognition and route ordering was performed, based on the Doors and People Test.[28] First, the

Doors Test Part A was administered, as a measure of non-spatial recognition. Afterwards, participants received nine cards of the presented pictures of doors, which were to be put in the right order. Again, two points were given for correctly ranked items, and one point for items that deviated one position. The recognition and temporal order components in this task resembled those of the Virtual Tübingen task, as the pictures were presented sequentially, one at a time. However, in contrast, this control task did not have a spatial component, since the pictures of the doors were not part of a route that could be represented mentally. Any discrepancy in performance between these two tests would thus be specific to spatial recognition or ordering processes, but not to temporal recognition or ordering processes.

Neuropsychological examination

All participants underwent a standardized neuropsychological examination, measuring three cognitive domains. 'Memory' was assessed by the immediate and delayed task of the Rey Auditory Verbal Learning Test[29] and the Visual Association Test.[30] 'Executive functioning' was assessed by the Trail Making Test - Part B[31] and the Stroop Color-Word Test card III.[32] 'Information processing speed' was assessed by the Trail Making Test part A.[31]

Statistical analysis

Between-group differences in population characteristics were analyzed with ANOVA for continuous variables, Mann-Whitney U tests for non-parametric data and chi-square tests for proportions.

Each spatial navigation subtest, each non-spatial control subtest (based on Doors task), and each neuropsychological test was standardized into z-scores, based on the means and standard deviations of controls. To obtain cognitive domain scores, the standardized scores of each neuropsychological test comprising that domain were then averaged into one composite score.

Between-group differences in navigation performance (standardized scores) on the three subtests were examined with ANCOVA, with age, sex, and education included as covariates. Next, within each group, the correlation was examined between each navigation subtest and functioning on the cognitive domains memory, executive functioning and information processing speed.

Table 1. Group characteristics

	Controls	aMCI	AD	P-value (three-way ANOVA)
n	43	33	41	
Age	74 ± 5	73 ± 9	78 ± 9	0.01 (controls = aMCI <AD)
Men	21 (49%)	13 (39%)	22 (54%)	0.47 (controls = aMCI = AD)
Education	5 (4-6)	4 (3.5-5.5)	5 (3-6)	0.44 (controls = aMCI = AD)
MMSE	28.4 ± 1.4	26.7 ± 3.4	23.3 ± 3.6	<0.01 (controls > aMCI > AD)[b]
Memory[a]	0.00 ± 0.82	-1.19 ± 1.10	-2.26 ± 1.17	<0.01 (controls > aMCI > AD)[b]
Executive functioning[a]	0.00 ± 0.70	-0.43 ± 0.87	-1.48 ± 1.10	<0.01 (controls > aMCI > AD)[b]
Information processing speed[a]	0.00 ± 1.00	-0.77 ± 1.78	-2.20 ± 3.03	<0.01 (controls > aMCI = AD)[b]

[a] Z-score ± standard deviation. Cognitive domains based on means and standard deviations of performance of controls.

[b] Analysis adjusted for age, sex, and education.

RESULTS

All three groups had similar gender distribution, chi^2 (2)=1.52, p=0.47, and education, Mann-Whitney U=638.50, p=0.44, but patients with AD were older than patients with aMCI and controls, F (2, 114)=5.10, MSE=58.53, p=0.01 (Table 1). As expected, global cognitive performance was best for controls, intermediate for patients with aMCI, and worst for patients with AD (MMSE, F(2, 114)=31.68, MSE=8.63, p<0.001; memory, F(2, 111) = 20.23, MSE=1.08, p<0.001; executive functioning, F(2, 111)=12.38, MSE=0.82, p<0.001; information processing speed, F(2, 109)=12.19, MSE = 3.49, p<0.001; Table 1).

Figure 2. Performance on spatial navigation subtests per group

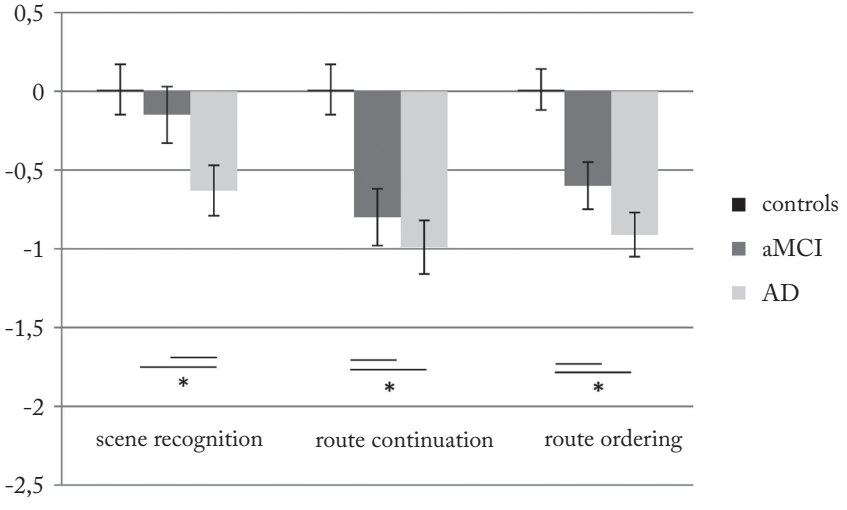


Figure 3. Performance on non-spatial subtests (based on Doors task) per group

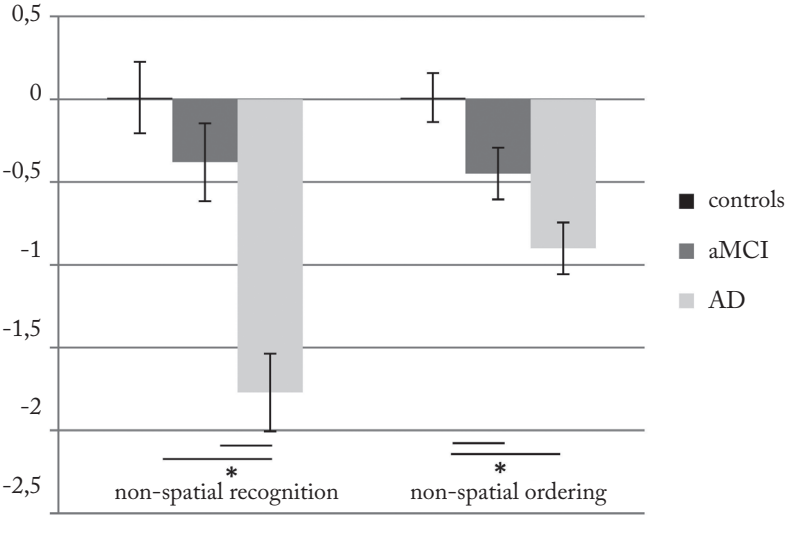


Table 2. Correlations between performance on spatial navigation subtests and functioning on three cognitive domains, within each group (Pearson's r)

Spatial navigation subtest	Cognitive domain
Scene recognition	Memory
	Executive functioning
	Information processing speed
Route continuation	Memory
	Executive functioning
	Information processing speed
Route ordering	Memory
	Executive functioning
	Information processing speed

[a] Adjusted for age, sex, and education.

There was a significant group effect for all three navigation subtests (scene recognition, F (2, 111)=4.59, MSE=1.03, p=0.001; route continuation, F (2, 111)=5.07, MSE=1.05, p <0.001; route ordering, F (2, 107)=6.01, MSE=0.68, p<0.001). For all subtests, performance was best for controls, intermediate for patients with aMCI, and worst for patients with AD, see Figure 2.

There was also a significant group effect for non-spatial recognition and ordering, based on the Doors task (non-spatial recognition, F (2, 105)=8.63, MSE=22.60, p 0.001; non-spatial ordering, F (2, 111)=3.88, MSE=0.93, p=0.003). For both tests, performance was best for controls, intermediate for patients with aMCI, and worst for patients with AD, see Figure 3. The most noticeable finding concerning these non-spatial control subtests was a particularly low performance of patients with AD in non-spatial recognition (Figure 3).

Of the three spatial subtests, patients with AD performed relatively best at scene recognition, although impaired relative to controls. In contrast, their impairments were larger on the two other subtests that require interaction with the environment. However, in a non-spatial task, performance of patients with AD was particularly poor for recognition.

Controls		aMCI		AD	
r[a]	p-value	r[a]	p-value	r[a]	p-value
0.30	**0.06**	0.14	0.46	**0.30**	**0.09**
0.25	0.13	0.13	0.51	-0.04	0.81
0.04	0.80	0.09	0.65	-0.07	0.70
0.18	0.28	0.29	0.13	0.19	0.30
0.21	0.20	0.07	0.72	**0.31**	**0.08**
-0.09	0.60	0.10	0.59	0.01	0.97
0.26	**0.10**	0.22	0.25	**0.36**	**0.04***
0.18	0.26	0.26	0.17	0.19	0.29
0.19	0.25	-0.13	0.49	0.23	0.19

Correlations per group between navigation subtests and cognitive domains are in the range of Pearson's r -0.13 to 0.36 (Table 2). The patterns of correlations appear to differ between the three groups, but the nature of these differences cannot be derived from these data.

DISCUSSION

In the present study we examined scene recognition, route continuation, and route ordering in cognitively normal older persons, patients with aMCI, and patients with AD. On all three navigation aspects, patients performed significantly worse than controls, and patients with aMCI were at an intermediate level between controls and patients with AD. This group difference was also found for non-spatial recognition and ordering. Relations between performance on spatial navigation and cognitive functioning on the domains memory, executive functioning, and information processing speed appeared to differ between groups.

This is the first study to assess scene recognition, route continuation, and route ordering in patients with aMCI or AD in a combined task design. The

fact that all three aspects are affected in patients with aMCI or AD provides valuable insight in the nature of navigation deficits that occur in these patients. Previous research has shown navigation deficits in patients with aMCI or AD similar to those found in the current study. Firstly, recognition of both spatial and non-spatial scenes was impaired in patients with AD, although patients with aMCI did not differ from controls. This extends previous findings on recognition. Impaired object recognition was found in patients with aMCI[33] and impaired recognition of spatial layouts was found in patients with aMCI or AD,[34–36] while landmark object recognition has been reported to be relatively spared in aMCI and AD.[10,34,37] This suggests that in patients with AD, simple features that are crucial for navigation can still be recognized as an automatic process, whereas recognition is impaired for complex spatial or for non-spatial configurations. In patients with AD, recognition impairment was most pronounced for non-spatial items, possibly because these items did not aid route processing and thus had little relevance. Secondly, route continuation was worse in patients with aMCI or AD compared to controls, but we did not find significant differences between the two patient groups. A few studies have investigated route continuation thus far and found similar deficits in patients with aMCI or AD.[11,14,33,36] Thirdly, patients with aMCI or AD performed worse than controls on a route ordering task, again our patient groups did not differ. This is in line with previously reported deficits in route ordering in patients with aMCI or AD.[10,38]

Differences in performance between patients with aMCI and AD warrant a closer look. In patients with aMCI recognition was intact, whereas in patients with AD recognition was reduced, especially non-spatial recognition. In both patient groups however, deficits were found for tasks that require elaborate processing of spatial information about the route. This distinction illustrates the non-unitary nature of navigation ability, as in patients with aMCI route processing can be selectively impaired while recognition is spared. Furthermore, this distinction may reflect differences in decay of the navigation aspects under study. Problems with complex spatial information processing may occur at an earlier phase in the disease process, already in the aMCI phase. Later on, after patients have converted to dementia, deficits in automatic processing of scenes emerge. Future studies are needed to further investigate these differential

trajectories of decline in navigation.

The current literature is inconclusive about the relation between navigation abilities and performance on standard neuropsychological tests. Some authors report that performance on cognitive tests assessing memory and executive functioning predict navigation performance,[11,36] whereas others did not fund such associations.[9,10] In our study, we did not observe a clear pattern of correlations between navigation subtests and the cognitive domains memory, executive functioning, and information processing speed within controls, patients with aMCI, or patients with AD. Nevertheless, associations appear to differ between groups, which may indicate a shift in strategy use with disease progression. However, no definite conclusions can be drawn based on our data. Future studies should investigate more precisely the relation between navigation abilities and functioning on cognitive domains, both in healthy older people and in patients with aMCI or AD. Knowledge about cognitive strategies in navigation may be applied to teach different cognitive approaches to patients who experience navigation difficulties.

Navigation is increasingly recognized as an important function for daily life, which is often affected in aging and in aMCI or AD. However, navigation assessment generally is not performed in current clinical practice. Given the importance of this function and the frequency of deficits, evaluation of navigation abilities should be included in standard neuropsychological examination in older populations. However, at present, no standardized navigation tasks are in use. Virtual reality tasks offer a good opportunity for this purpose, as they correlate well with real life navigation performance.[14,39] In this context, the Virtual Tübingen is of interest, as it measures three important aspects of spatial navigation and is easy to administer. It can thus be applied in both research settings and clinical practice.

Furthermore, navigation assessment has been proposed to aid in differential diagnosis between normal aging, MCI subtypes and AD, or in predicting which patients with aMCI will convert to dementia.[6,7,9,11,33,38]

In conclusion, multiple aspects of navigation are affected in patients with aMCI, and, to a larger extent, in patients with AD. Assessment of navigation abilities in older populations should be part of standard clinical practice.

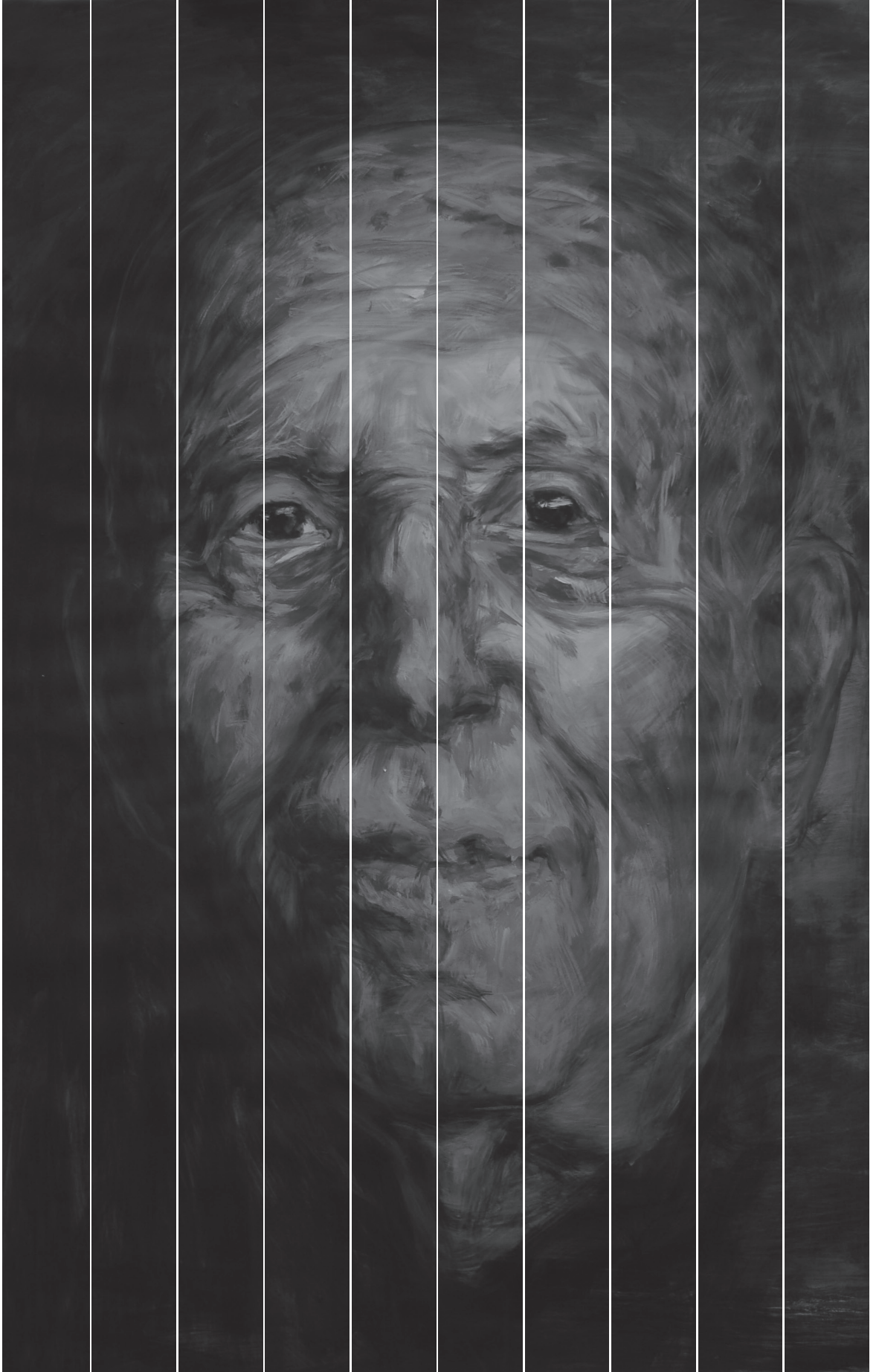
REFERENCES

1. Pai M, Jacobs WJ. Topographical disorientation in community-residing patients with Alzheimer's disease. *International journal of geriatric psychiatry*. 2004;19:250–5.
2. Wolbers T, Hegarty M. What determines our navigational abilities? *Trends in cognitive sciences*. 2010;14:138–46.
3. Burgess N. Spatial memory: how egocentric and allocentric combine. *Trends in cognitive sciences*. 2006;10:551–7.
4. Harris MA, Wolbers T. Aging effects on path integration and landmark navigation. *Hippocampus*. 2012;22:1770–80.
5. Harris MA, Wiener JM, Wolbers T. Aging specifically impairs switching to an allocentric navigational strategy. *Frontiers in aging neuroscience*. 2012;4:29.
6. Hort J, Laczó J, Vyhnálek M, Bojar M, Bures J, Vlcek K. Spatial navigation deficit in amnestic mild cognitive impairment. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104:4042–7.
7. Laczó J, Andel R, Vlček K, Macoška V, Vyhnálek M, Tolar M, Bojar M, Hort J. Spatial navigation and APOE in amnestic mild cognitive impairment. *Neurodegenerative diseases*. 2011;8:169–77.
8. Weniger G, Ruhleder M, Lange C, Wolf S, Irle E. Egocentric and allocentric memory as assessed by virtual reality in individuals with amnestic mild cognitive impairment. *Neuropsychologia*. 2011;49:518–27.
9. Monacelli AM, Cushman LA, Kavcic V, Duffy CJ. Spatial disorientation in Alzheimer's disease: the remembrance of things passed. *Neurology*. 2003;61:1491–7.
10. DeIpolyi AR, Rankin KP, Mucke L, Miller BL, Gorno-Tempini ML. Spatial cognition and the human navigation network in AD and MCI. *Neurology*. 2007;69:986–97.
11. Pengas G, Patterson K, Arnold RJ, Bird CM, Burgess N, Nestor PJ. Lost and found: bespoke memory testing for Alzheimer's disease and semantic dementia. *Journal of Alzheimer's disease*. 2010;21:1347–65.
12. Lithfous S, Dufour A, Després O. Spatial navigation in normal aging and the prodromal stage of Alzheimer's disease: Insights from imaging and behavioral studies. *Aging research reviews*. 2012;12:201–213.
13. Mapstone M, Steffenella TM, Duffy CJ. A visuospatial variant of mild cognitive impairment: getting lost between aging and AD. *Neurology*. 2003;60:802–8.

14. Cushman LA, Stein K, Duffy CJ. Detecting navigational deficits in cognitive aging and Alzheimer disease using virtual reality. *Neurology*. 2008;71:888–95.
15. Laczó J, Vlcek K, Vyhnálek M, Vajnerová O, Ort M, Holmerová I, Tolar M, Andel R, Bojar M, Hort J. Spatial navigation testing discriminates two types of amnestic mild cognitive impairment. *Behavioural brain research*. 2009;202:252–9.
16. Maguire EA, Burgess N, Donnett JG, Frackowiak RS, Frith CD, O'Keefe J. Knowing where and getting there: a human navigation network. *Science*. 1998;280:921–4.
17. Burgess N, Maguire EA, O'Keefe J. The human hippocampus and spatial and episodic memory. *Neuron*. 2002;35:625–41.
18. Retailleau A, Etienne S, Guthrie M, Boraud T. Where is my reward and how do I get it? Interaction between the hippocampus and the basal ganglia during spatial learning. *Journal of physiology*. 2011;106:72–80.
19. Serino S, Riva G. Getting lost in Alzheimer's disease: a break in the mental frame syncing. *Medical hypotheses*. 2013;80:416–21.
20. Epstein RA, Higgins JS, Jablonski K, Feiler AM. Visual scene processing in familiar and unfamiliar environments. *Journal of neurophysiology*. 2007;97:3670–83.
21. Epstein R, Kanwisher N. A cortical representation of the local visual environment. *Nature*. 1998;392:6–9.
22. Raz N, Ghisletta P, Rodrigue KM, Kennedy KM, Lindenberger U. Trajectories of brain aging in middle-aged and older adults: regional and individual differences. *NeuroImage*. 2010;51:501–11.
23. Wiener JM, Kmecova H, de Condappa O. Route repetition and route retracing: effects of cognitive aging. *Frontiers in aging neuroscience*. 2012;4:7.
24. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Archives of neurology*. 1999;56:303–8.
25. 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;34:939–44.
26. Van der Ham IJM, van Zandvoort MJE, Meilinger T, Bosch SE, Kant N, Postma A. Spatial and temporal aspects of navigation in two neurological patients. *NeuroReport*. 2010;21:685–9.

27. Veen H van, Distler H. Navigating through a virtual city: Using virtual reality technology to study human action and perception. *Future Generation Computer Sciences*. 1998;14:231–242.
28. Baddeley AD, Emslie H, Nimmo-Smith I. *Doors and People: A Test of Visual and Verbal Recall and Recognition*. 1994; Bury St. Edmunds, England: Thames Valley Test.
29. Van der Elst W, van Boxtel MPJ, van Breukelen GJP, 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*. 2005;11:290–302.
30. Lindeboom J, Schmand B, Tulner L, Walstra G, Jonker C. Visual association test to detect early dementia of the Alzheimer type. *Journal of neurology, neurosurgery, and psychiatry*. 2002;73:126–33.
31. Corrigan JD, Hinkeldey NS. Relationships between parts A and B of the Trail Making Test. *Journal of clinical psychology*. 1987;43:402–9.
32. Stroop J. Studies of interference in serial verbal reactions. *Journal of Experimental Psychology*. 1935;18:643–662.
33. Mitolo M, Gardini S, Fasano F, Crisi G, Pelosi A, Pazzaglia F, Caffarra P. Visuospatial memory and neuroimaging correlates in mild cognitive impairment. *Journal of Alzheimer's disease*. 2013;35:75–90.
34. Cherrier MM, Mendez M, Perryman K. Route learning performance in Alzheimer disease patients. *Neuropsychiatry, neuropsychology, and behavioral neurology*. 2001;14:159–68.
35. Bird CM, Chan D, Hartley T, Pijnenburg YA, Rossor MN, Burgess N. Topographical short-term memory differentiates Alzheimer's disease from frontotemporal lobar degeneration. *Hippocampus*. 2010;20:1154–69.
36. Benke T, Karner E, Petermichl S, Prantner V, Kemmler G. Neuropsychological Deficits Associated With Route Learning in Alzheimer Disease, MCI, and Normal Aging. *Alzheimer disease and associated disorders*. 2013;00:1–6.
37. Kessels RPC, van Doormaal A, Janzen G. Landmark recognition in Alzheimer's dementia: spared implicit memory for objects relevant for navigation. *PloS one*. 2011;6:e18611.
38. Bellassen V, Iglói K, de Souza LC, Dubois B, Rondi-Reig L. Temporal order memory assessed during spatiotemporal navigation as a behavioral cognitive marker for differential Alzheimer's disease diagnosis. *The Journal of neuroscience*. 2012;32:1942–52.

39. Richardson AE, Montello DR, Hegarty M. Spatial knowledge acquisition from maps and from navigation in real and virtual environments. *Memory & cognition*. 1999;27:741–50.



CHAPTER 10

GENERAL DISCUSSION

The studies in this thesis focus on microvascular disease in relation to cognitive functioning and dementia. Our results show that various systemic biomarkers of microvascular disease are related to subtle cognitive decrements in the general older population. We also show that cerebral biomarkers of microvascular disease are prevalent in patients with amnestic mild cognitive impairment (aMCI) or early Alzheimer's disease (AD), and are related to co-existing vascular brain pathologies. However, in these patients, the association between cerebral markers of microvascular disease and cognitive functioning is less strong than the associations reported in the general population.

Biomarkers in Alzheimer's disease and vascular cognitive impairment

The term "biomarker" ("biological marker", see box) refers to a broad subcategory of medical signs, that is, objective indications of medical state observed from outside the patient, which can be measured accurately and reproducibly.[1,2] Biomarkers may be used in clinical trials to establish "proof of concept" for benefit of novel treatments. Other applications include identification of those patients with a disease or abnormal condition, staging of disease, indication of disease prognosis, or prediction and monitoring of clinical response to an intervention.[2]

Biomarkers have received much attention in dementia and AD research. Biomarkers that have been identified in AD include amyloid β in the cerebral spinal fluid (CSF), amyloid within the brain as measured with positron emission tomography (PET) imaging, increased concentrations of CSF total tau (t-tau) and phosphorylated tau (p-tau), hypometabolism on fluorodeoxyglucose (FDG) PET imaging, and atrophy on structural magnetic resonance imaging (MRI).[3,4]

Trials in patients with AD that have used amyloid modifying therapy indicate that indeed the disease process may be influenced, as is reflected in clearance of amyloid plaques in the brain.[5] However, this was not accompanied by an improvement of clinical symptoms, such as less cognitive decline.[5] Therefore, it has been argued that such treatments should be given earlier in the disease process to be effective. However, specifically in early stages of the disease, it is difficult to reliably identify people with pathology. This stimulated the field of AD research in the pursuit of biomarkers, especially those markers relating to

Box: Biomarkers and other characteristics

(based on Biomarkers Definitions Working Group 2001[2] and Lassere et al.[36,37])

To understand the value and usefulness of the markers of microvascular disease that are described in this thesis, it is important to distinguish between biomarkers, clinical endpoints, surrogate endpoints, risk factors, and prognostic factors.

A *biological marker* (biomarker) is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Such a variable may be a biochemical marker, a cellular marker, a cytokine marker, a genetic marker, an imaging marker, or a physiological marker. Biomarkers may be used in clinical trials to establish "proof of concept" (etiological value). Other applications include a diagnostic tool for identification of those patients with a disease or abnormal condition, a tool for staging of disease, an indicator of disease prognosis, or prediction and monitoring of clinical response to an intervention.

A *clinical endpoint* is a characteristic or variable that reflects how a patient feels, functions or survives.

A *surrogate endpoint* is a biomarker that is intended to substitute for a clinical endpoint. It is expected to predict clinical benefit based (or harm or lack of benefit or harm) on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence.

A *risk factor* is a characteristic that is present in individuals without disease who are more likely to develop a certain outcome. A risk factor may or may not be a biomarker.

A *prognostic factor* is similar to a risk factor, except that it is present in individuals who have a disease. Prognostic factors can be used to identify subgroups that are more likely to respond to treatment.

Before a biomarker can reach the status of surrogate endpoint, treatment studies are required, showing that a treatment-associated change in the surrogate is accompanied by related change in target outcome. It is important to realize that treatment of a single biomarker may capture only a portion, or none, of the treatment effect. Furthermore, the treatment that modifies the surrogate may have unsuspected unanticipated adverse consequences for the patient, or may reduce the association between surrogate and target outcome.

early pathophysiological processes before the onset of cognitive decline.[3,4] These biomarkers can be applied both as tools for identification of a target group for treatment and as measures of treatment effects. The ultimate anticipated effect in these studies is the delay or slowing down of cognitive deterioration.

In this light, biomarkers that accurately reflect the vascular burden in dementia also deserve attention. Since cerebrovascular disease is an important contributor to the clinical and cognitive profile in dementia,[6] biomarkers of vascular damage may be closely related to cognitive functioning and may thus have high scientific and clinical relevance.

Systemic markers of microvascular disease

In the first part of this thesis, we investigated measures of microvascular disease in parts of the body outside the brain and the relation with cognitive functioning, in older individuals. In chapter 2, an overview is given of the literature on small blood vessels in the retina in this regard. Retinal microvascular changes were related to dementia, to modest decrements in cognitive functioning in non-demented individuals, and to brain imaging abnormalities. Some studies did not find a significant association between presence of retinopathy and cognitive functioning, as was the case in our own population-based study of community-dwelling older persons without severe cognitive problems (chapter 5). Nevertheless, in this same cohort, we did find associations between other systemic markers of microvascular disease and cognitive functioning. Chapter 3 describes that albuminuria, a marker of renal microvascular damage, was associated with modest cognitive decrements 5-7 years later. Furthermore, serum biomarkers of low-grade inflammation and endothelial dysfunction were cross-sectionally related to cognitive decrements (chapter 4).

Microvascular disease is important to the brain

Consistent associations are found between systemic markers of microvascular disease and worse cognitive functioning and brain abnormalities in the aging population. This association is already present in normally functioning older people without cognitive complaints, which implies that these markers represent early pathological processes leading to cognitive deterioration. We have found some indications that those domains that appear to be most affected

are more related to executive functioning/subcortical functions rather than to memory functioning, a distinction that has been reported before in populations with vascular damage.[7]

The relations between different systemic biomarkers of microvascular disease and cognitive functioning may convey different messages about causality. Albuminuria and retinal vascular changes are only indirectly related to cognitive functioning, as damage to the kidney or to the retina is unlikely to play a causative role in cognitive decline. Rather, presence of these markers reflects vascular pathophysiological processes that are considered to be of importance for cognitive functioning. On the other hand, biomarkers of low-grade inflammation and endothelial dysfunction were found to mediate the association between vascular risk factors and cognitive impairment. This suggests that low-grade inflammation and endothelial dysfunction may take part in the etiological cascade of subclinical vascular damage eventually leading to cognitive impairment.

Overall, the hypothesis was confirmed that microvascular disease elsewhere in the body is important to the brain,[8] as reflected in consistent associations between microvascular damage and cognitive functioning.

Not a marker of reduced cognitive performance

The association with cognitive functioning in all studies was rather weak, which has several possible explanations and implications. First, these markers are also related to an increased risk of mortality.[9–11] This may lead to a bias in survivors, as non-survivors may have died before they reach the point of developing cognitive impairment. Second, in most older persons, microvascular damage is not the single pathology. Other underlying pathologies of cognitive impairment, such as neurodegenerative processes, are also likely to be present, which may obscure the relation between microvascular pathology and cognitive deficits. Finally, prospective population-based studies, such as ours, have the methodological limitation that persons with a less favorable vascular risk factor profile at baseline or with severe cognitive dysfunction are more likely to be lost to follow-up.

Therefore, despite their relation with cognitive functioning, and despite their easy way of being measured, these biomarkers of systemic microvascular disease are of limited value as markers of reduced cognitive performance in the population.

Cerebral markers of microvascular disease

In the second part of this thesis, we investigated measures of microvascular disease in the brain in patients with aMCI or early AD. Chapter 6 describes that multiple microbleeds were associated with disruptions of the microstructure and organization of the cerebral network as measured with diffusion tensor imaging (DTI), independent of other measures of small vessel disease or atrophy. In chapter 7, 7 Tesla MRI was used to investigate the presence of microbleeds in detail. We showed that the majority of patients with aMCI or early AD exhibit cerebral microbleeds. In chapter 8, we used 7 Tesla MRI to image cortical microinfarcts. We found that numbers were not significantly increased in patients relative to controls, but microinfarcts did appear to be related to cerebral microbleed numbers and to other, larger, cortical infarcts.

Markers of presence of brain pathology, rather than disease severity

In our studies with patients with aMCI or early AD, the numbers of microbleeds or microinfarcts were not associated with cognitive functioning. For microbleeds, this is in line with previous literature about AD.[12–17] Only one previous study reported an association with cognitive impairment in patients with AD, but this concerned patients with eight or more microbleeds.[18] In contrast, in non-demented individuals from population-based cohorts, both the presence and number of microbleeds were associated with impaired cognition.[19–22] Yet, we showed a relation with dementia: microbleeds were more prevalent in patients with aMCI or early AD compared to controls (chapter 7). Similarly, microinfarcts in neuropathology studies were more prevalent in people who died with dementia than in people who died without dementia.[23] One in vivo study found that microinfarcts were more prevalent in patients with AD, and were related with cognitive functioning.[24] In our own in vivo data, however, we found no difference in microinfarct prevalence between patients with aMCI or early AD and controls, and no relation with cognitive functioning. A possible explanation is that microinfarcts develop as dementia progresses. This would imply that they are more prevalent in end-stage dementia, as can be found in autopsy studies, whereas our patient population was still at an early stage of the disease. More data from larger groups and at various disease stages (both pre-clinical and more advanced) are needed to further unravel the relation between

cortical microinfarcts and cognitive functioning and dementia.

In sum, cerebral microvascular lesions are associated with (end-stage) dementia presence, and with cognitive functioning in the general population but not so much in patients with AD. This implies that microbleeds and microinfarcts are primarily markers of presence of brain pathology underlying cognitive impairment, rather than of its severity. Therefore, in the general population, the presence of cerebral microvascular lesions might distinguish between individuals with or without early stages of a dementia process, possibly due to AD or vascular damage. In patients with a clinical diagnosis of early AD, however, only very high lesion counts that are present in a small subgroup may be indicative for more severe pathology or a different disease profile.

Novel MRI techniques

Our studies demonstrate the potential of novel MRI techniques in studying cerebral markers of vascular causes of cognitive impairment. With high quality DTI, we are able to detect very subtle changes in the white matter microstructure and organization that cannot be assessed with conventional imaging techniques. These changes, such as cerebral network disturbances, do appear to have clinical importance as they are related to cognitive decrements.[25] Furthermore, the use of 7 Tesla MRI compared to 1.0-3.0 Tesla MRI allows for a high spatial resolution and increased detection rates for microbleeds.[26] With 7 Tesla MRI, it is possible for the first time to assess microinfarcts in vivo.[27] Thus, ultra-high field imaging appears to reflect the ubiquity of these lesions as observed in neuropathology studies.[28] By applying these techniques, we took a first step in assessing the microvascular burden in patients with aMCI or early AD in high detail. This can help understanding pathologies leading to cognitive impairment. For example, microbleeds that previously would have remained undetected can now be visualized. This may imply that these microbleeds reflect other vascular pathologies than those that are also observed at lower field strength. Another application of microbleeds in the study of AD is to serve as biomarkers in therapeutic trials in AD. Presence of multiple microbleeds prior to therapy is considered to reflect an increased risk of treatment-related complications such as risk of vasogenic edema or hemorrhagic stroke.[29,30] Furthermore, microinfarcts can now be studied in prospective studies. This enables investigation of their

development and of their value in predicting clinical and cognitive outcomes.

There are also some limitations of 7 Tesla MRI, compared to lower field strengths. Stringent contraindications, such as metal implants or claustrophobia, may cause a selection bias. Furthermore, long acquisition times are used to obtain a high spatial resolution, making images more susceptible to movement artifacts. Such artifacts are an important drawback because they complicate the assessment of small lesions such microbleeds or microinfarcts. In addition, since patients with aMCI or early AD typically have more trouble laying still in the scanner than healthy controls, these artifacts may cause a bias for visual rating of scans. Finally, 7 Tesla MRI scanners are not widely available, which limits their use in standard clinical practice or as a population screening instrument.

A specific cognitive deficit in patients with aMCI or AD

In early dementia, various cognitive functions can deteriorate. Disturbances in memory and executive functions are most pronounced. A cognitive function that has received less attention, but is also often affected in patients with aMCI or early AD, is spatial navigation. Deficits in spatial navigation are consistently found in this patient population and highly impact daily life. In the third part of this thesis, chapter 9, we investigated three fundamental aspects of spatial navigation: scene recognition, route continuation, and route ordering. Patients performed worse than controls, and patients with aMCI were at an intermediate level between controls and patients with AD. We showed that spatial navigation is a non-unitary process, and different aspects vary in their trajectories of decay in patients with aMCI or early AD. Because of the clinical relevance of spatial navigation deficits, we argue that assessment of navigation abilities in older populations should be part of standard clinical practice. Virtual reality tasks are a valid and feasible instrument for this purpose.[31]

Directions for future research and implications for clinical care

Concerning systemic microvascular markers in the general population, most studies are published on cross-sectional data. However, from studies in this thesis and from the literature, indications arise that concurrent and prospective associations between these markers and cognitive functioning may differ. It is currently unknown at what stage in the etiological cascade of cognitive decline

microvascular pathology occurs. Microvascular disease may precede clinical symptoms of dementia or cognitive impairment, or rather may emerge at the same time, as a relatively late phenomenon. Longitudinal studies in large cohorts should elucidate this and establish the prognostic value of microvascular markers in the population.

Appreciation of vascular disease as a cause of cognitive decline and dementia has led to trials aimed at influencing vascular risk factors, with cognitive decline or dementia as an outcome. Results, however, have not convincingly shown that treatment of vascular risk factors can actually prevent or postpone cognitive decline and dementia.[32] A possible explanation is that treatment was given too late, and was not effective once cognitive decline has set in. Exposure to vascular risk from midlife is related to late-life cognitive impairment,[33] therefore future studies should focus on targeting vascular risk factors in midlife. Nevertheless, also in older age groups, vascular treatment may still reduce small vessel disease and thus may help prevent dementia.[34] These complex relations between vascular risk, treatment, and cognitive decline and dementia need to be studied prospectively, across the age span.

The novel imaging techniques that were used in this thesis open the door for many studies on the vascular burden in AD. With ultra-high field strength MRI, microbleeds and microinfarcts can be measured better than ever before. These techniques can now be applied in larger prospective studies, including in pre-clinical and more advanced stages of the disease, to investigate the development and prognostic and etiologic relevance of cerebral microvascular biomarkers in AD. Furthermore, findings on 7 Tesla MRI should be translated to more widely available techniques, such as 3 Tesla MRI, in order to implement knowledge in a clinical setting. Another interesting challenge is to combine techniques to investigate how large numbers of microbleeds, or microinfarcts, as detected with 7 Tesla MRI, are related to the white matter microstructure on DTI. Perhaps, with these techniques, future studies will identify new biomarkers for cognitive decline. This would be exciting, as currently a total of 41% of the variation in cognitive decline can be explained by known pathologic indices of AD, cerebrovascular disease, and Lewy body disease, while the remaining 59% remains unexplained.[35]

An important message from our studies is that small vessel disease,

and not only large vessel disease, is important for the brain and for cognitive functioning. Persons without overt cognitive problems who show signs of microvascular disease, such as albuminuria, retinopathy, or low-grade inflammation/endothelial dysfunction, may be at risk of developing cognitive impairment. Clinicians should be alert for cognitive complaints in persons with macro and microvascular disease. Furthermore, modification of vascular risk factors should start early. When treatment is started in midlife, this may help to delay dementia onset. In patients with dementia, the clinical profile is likely to be determined by both neurodegenerative and cerebrovascular disease. Therefore, treatment should focus on both pathologies.

Conclusion

Microvascular disease is important with regard to cognition. An association between markers of microvascular disease and cognitive functioning is present in people without cognitive complaints, although this association is rather weak. Therefore, markers at the population level are not suitable for detection of individuals at risk for cognitive decline. In patients with aMCI or early AD, cerebral markers of microvascular disease are prevalent and are related to other vascular brain damage, but the association with cognitive functioning and clinical variables needs further study.

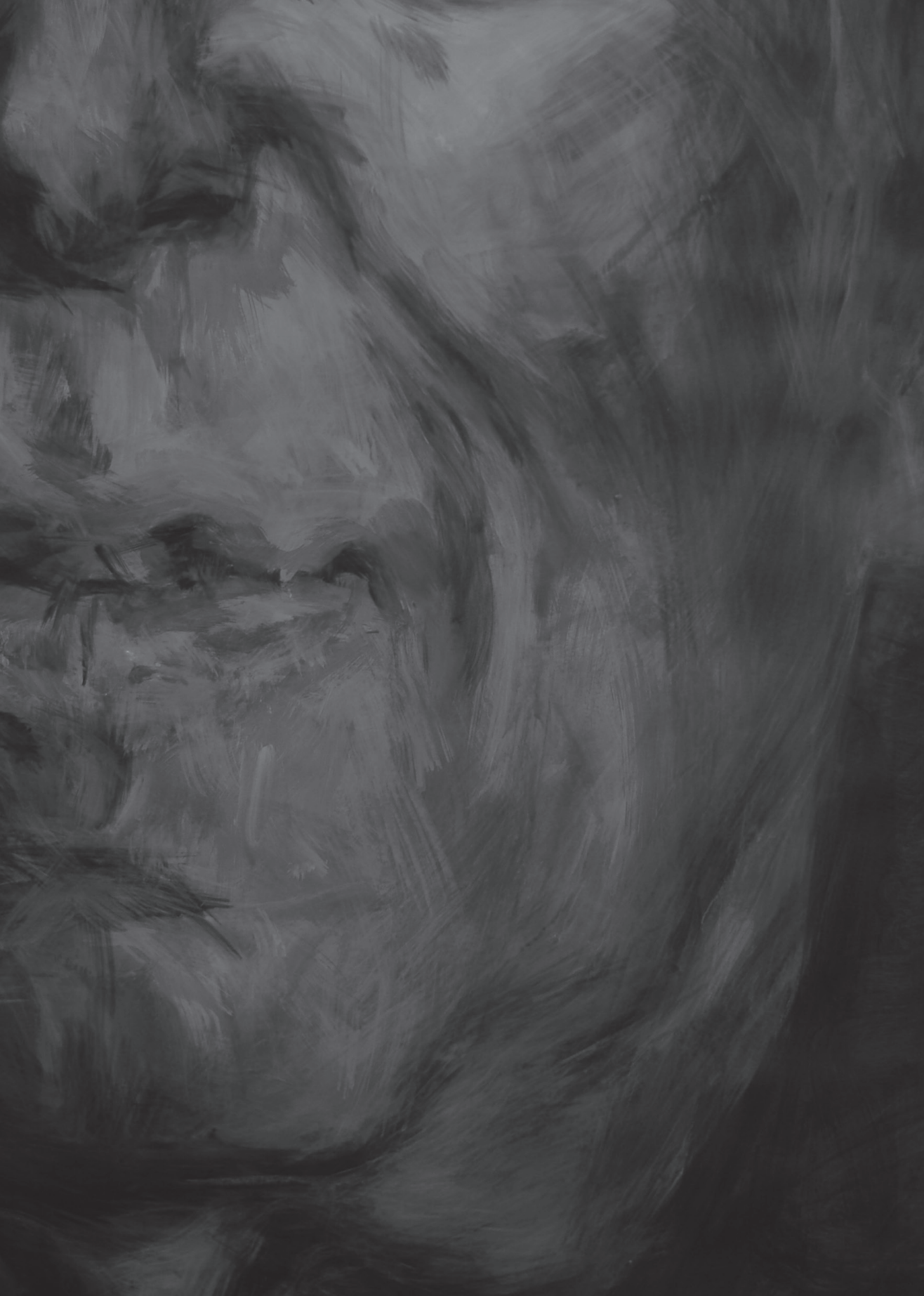
REFERENCES

1. Strimbu K, Tavel JA. What are biomarkers? *Current opinion in HIV and AIDS*. 2010;5:463–6.
2. Biomarkers Definition Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clinical pharmacology and therapeutics*. 2001;69:89–95.
3. Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet neurology*. 2010;9:119–28.
4. Jack CR, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, Shaw LM, Vemuri P, Wiste HJ, Weigand SD, Lesnick TG, Pankratz VS, Donohue MC, Trojanowski JQ. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet neurology*. 2013;12:207–16.
5. Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, Nicoll JAR. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet*. 2008;372:216–23.
6. O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, Bowler J V, Ballard C, DeCarli C, Gorelick PB, Rockwood K, Burns A, Gauthier S, DeKosky ST. Vascular cognitive impairment. *Lancet neurology*. 2003;2:89–98.
7. Prins ND, van Dijk EJ, den Heijer T, Vermeer SE, Jolles J, Koudstaal PJ, Hofman A, Breteler MMB. Cerebral small-vessel disease and decline in information processing speed, executive function and memory. *Brain*. 2005;128:2034–41.
8. Thompson CS, Hakim AM. Living beyond our physiological means: small vessel disease of the brain is an expression of a systemic failure in arteriolar function: a unifying hypothesis. *Stroke*. 2009;40:e322–30.
9. Wong TY, McIntosh R. Hypertensive retinopathy signs as risk indicators of cardiovascular morbidity and mortality. *British medical bulletin*. 2005;73-74:57–70.
10. Nitsch D, Grams M, Sang Y, Black C, Cirillo M, Djurdjev O, Iseki K, Jassal SK, Kimm H, Kronenberg F, Oien CM, Levey AS, Levin A, Woodward M, Hemmelgarn BR. Associations of estimated glomerular filtration rate and albuminuria with mortality and renal failure by sex: a meta-analysis. *BMJ*. 2013;346:f324.

11. Jager A, van Hinsbergh VW, Kostense PJ, Emeis JJ, Yudkin JS, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Stehouwer CD. von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and nondiabetic subjects: the Hoorn Study. *Arteriosclerosis, thrombosis, and vascular biology*. 1999;19:3071–8.
12. Pettersen JA, Sathiyamoorthy G, Gao F-Q, Szilagyi G, Nadkarni NK, St George-Hyslop P, Rogaeva E, Black SE. Microbleed topography, leukoaraiosis, and cognition in probable Alzheimer disease from the Sunnybrook dementia study. *Archives of neurology*. 2008;65:790–5.
13. Cordonnier C, van der Flier WM, Sluimer JD, Leys D, Barkhof F, Scheltens P. Prevalence and severity of microbleeds in a memory clinic setting. *Neurology*. 2006;66:1356–60.
14. Hanyu H, Tanaka Y, Shimizu S, Takasaki M, Abe K. Cerebral microbleeds in Alzheimer's disease. *Journal of neurology*. 2003;250:1496–7.
15. Nakata Y, Shiga K, Yoshikawa K, Mizuno T, Mori S, Yamada K, Nakajima K. Subclinical brain hemorrhages in Alzheimer's disease: evaluation by magnetic resonance T2*-weighted images. *Annals of the New York Academy of Sciences*. 2002;977:169–72.
16. Nakata-Kudo Y, Mizuno T, Yamada K, Shiga K, Yoshikawa K, Mori S, Nishimura T, Nakajima K, Nakagawa M. Microbleeds in Alzheimer disease are more related to cerebral amyloid angiopathy than cerebrovascular disease. *Dementia and geriatric cognitive disorders*. 2006;22:8–14.
17. Van der Vlies AE, Goos JDC, Barkhof F, Scheltens P, van der Flier WM. Microbleeds do not affect rate of cognitive decline in Alzheimer disease. *Neurology*. 2012;79:763–9.
18. Goos JDC, Kester MI, Barkhof F, Klein M, Blankenstein MA, Scheltens P, van der Flier WM. Patients with Alzheimer disease with multiple microbleeds: relation with cerebrospinal fluid biomarkers and cognition. *Stroke*. 2009;40:3455–60.
19. Poels MMF, Ikram MA, van der Lugt A, Hofman A, Niessen WJ, Krestin GP, Breteler MMB, Vernooij MW. Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. *Neurology*. 2012;78:326–33.
20. Yakushiji Y, Nishiyama M, Yakushiji S, Hirotsu T, Uchino A, Nakajima J, Eriguchi M, Nanri Y, Hara M, Horikawa E, Kuroda Y. Brain microbleeds and global cognitive function in adults without neurological disorder. *Stroke*. 2008;39:3323–8.

21. Yakushiji Y, Noguchi T, Hara M, Nishihara M, Eriguchi M, Nanri Y, Nishiyama M, Hirotsu T, Nakajima J, Kuroda Y, Hara H. Distributional impact of brain microbleeds on global cognitive function in adults without neurological disorder. *Stroke*. 2012;43:1800–5.
22. Takashima Y, Mori T, Hashimoto M, Kinukawa N, Uchino A, Yuzuriha T, Yao H. Clinical correlating factors and cognitive function in community-dwelling healthy subjects with cerebral microbleeds. *Journal of stroke and cerebrovascular diseases*. 2011;20:105–10.
23. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesions. *Lancet neurology*. 2012;11:272–82.
24. Van Rooden S, Goos JDC, van Opstal AM, Versluis MJ, Webb AG, Blauw GJ, van der Flier WM, Scheltens P, Barkhof F, van Buchem MA, van der Grond J. Increased Number of Microinfarcts in Alzheimer Disease at 7-T MR Imaging. *Radiology*. 2013;
25. Reijmer YD, Leemans A, Caeyenberghs K, Heringa SM, Koek HL, Biessels GJ. Disruption of cerebral networks and cognitive impairment in Alzheimer disease. *Neurology*. 2013;80:1370–7.
26. Conijn MMA, Geerlings MI, Biessels GJ, Takahara T, Witkamp TD, Zwanenburg JJM, Luijten PR, Hendrikse J. Cerebral microbleeds on MR imaging: comparison between 1.5 and 7T. *American journal of neuroradiology*. 2011;32:1043–9.
27. Van Veluw SJ, Zwanenburg JJM, Engelen-Lee J, Spliet WGM, Hendrikse J, Luijten PR, Biessels GJ. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. *Journal of cerebral blood flow and metabolism*. 2013;33:322–9.
28. Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. *Journal of neural transmission*. 2002;109:813–36.
29. Cordonnier C, van der Flier WM. Brain microbleeds and Alzheimer's disease: innocent observation or key player? *Brain*. 2011;134:335–44.
30. Sperling RA, Jack CR, Black SE, Frosch MP, Greenberg SM, Hyman BT, Scheltens P, Carrillo MC, Thies W, Bednar MM, Black RS, Brashear HR, Grundman M, Siemers ER, Feldman HH, Schindler RJ. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials : Recommendations from the Alzheimer's Association Research Roundtable Workgroup. *Alzheimer's & Dementia*. 2011;7:367–385.

31. Cushman LA, Stein K, Duffy CJ. Detecting navigational deficits in cognitive aging and Alzheimer disease using virtual reality. *Neurology*. 2008;71:888–95.
32. Richard E, Moll van Charante EP, van Gool WA. Vascular risk factors as treatment target to prevent cognitive decline. *Journal of Alzheimer's disease*. 2012;32:733–40.
33. Reijmer YD, van den Berg E, Dekker JM, Nijpels G, Stehouwer CDA, Kappelle LJ, Biessels GJ. Development of vascular risk factors over 15 years in relation to cognition: the Hoorn Study. *Journal of the American Geriatrics Society*. 2012;60:1426–33.
34. Sörös P, Whitehead S, Spence JD, Hachinski V. Antihypertensive treatment can prevent stroke and cognitive decline. Nature reviews. *Neurology*. 2013;9:174–8.
35. Boyle PA, Wilson RS, Yu L, Barr AM, Honer WG, Schneider JA, Bennett DA. Much of late life cognitive decline is not due to common neurodegenerative pathologies. *Annals of neurology*. 2013;73:478-489.
36. Lassere MN, Johnson KR, Boers M, Tugwell P, Brooks P, Simon L, Strand V, Conaghan PG, Ostergaard M, Maksymowych WP, Landewe R, Bresnihan B, Tak P-P, Wakefield R, Mease P, Bingham CO, Hughes M, Altman D, Buyse M, Galbraith S, Wells G. Definitions and validation criteria for biomarkers and surrogate endpoints: development and testing of a quantitative hierarchical levels of evidence schema. *The Journal of rheumatology*. 2007;34:607–15.
37. Lassere MN. The Biomarker-Surrogacy Evaluation Schema: a review of the biomarker-surrogate literature and a proposal for a criterion-based, quantitative, multidimensional hierarchical levels of evidence schema for evaluating the status of biomarkers as surrogate endp. *Statistical methods in medical research*. 2008;17:303–40.



SUMMARY

Cognitive impairment and dementia form a major health issue, affecting a considerable proportion of the aging population. Cerebral vascular damage is increasingly recognized as one of the main causes of cognitive decline in aging and dementia. Another main cause of cognitive deterioration in older people are neurodegenerative processes, resulting in Alzheimer's disease (AD) type pathologies. Evidence is growing for a crucial role of vascular damage in AD. For instance, vascular risk factors contribute to development of AD. Furthermore, on autopsy, a majority of older people exhibit a mixed profile of vascular damage and AD pathology. These findings have led to a dimensional view on cognitive decline and dementia, with a spectrum ranging from patients with pure vascular dementia to patients with pure AD and including a large majority of patients with contributions from both pathologies. A current challenge is to explore the role of vascular disease in causing cognitive impairment, by itself or in interaction with AD.

The brain may be affected by damage to both large and small blood vessels. The cerebral microvasculature is fundamentally important for brain functioning, and microvascular damage is important for the entire range of cognitive functioning, from subtle decrements in normal aging to mild cognitive impairment (MCI) and frank dementia. The research in this thesis focused on microvascular disease. We aimed to investigate the relation between biomarkers of microvascular disease and cognitive functioning, in the general older population using systemic markers (part I) and in patients with amnestic mild cognitive impairment (aMCI) or early AD using novel brain magnetic resonance imaging (MRI) techniques (part II). Finally, we investigated a cognitive function that is often impaired in patients with aMCI or early AD (part III).

Part I – Systemic markers of microvascular disease and cognitive functioning

In the first part of this thesis, we investigated measures of microvascular disease in parts of the body outside the brain and the relation with cognitive functioning, in older individuals. In **chapter 2**, an overview is given of the literature on

small blood vessels in the retina in this regard. Retinal microvascular changes were related to dementia, to modest decrements in cognitive functioning in non-demented individuals, and to brain imaging abnormalities. Some studies did not find a significant association between presence of retinopathy and cognitive functioning, as was the case in our own population-based study of community-dwelling older persons without severe cognitive problems (**chapter 5**). Nevertheless, in this same cohort, we did find associations between other systemic markers of microvascular disease and cognitive functioning. **Chapter 3** describes that albuminuria, a marker of renal microvascular damage, was associated with modest cognitive decrements 5-7 years later. Furthermore, serum biomarkers of low-grade inflammation and endothelial dysfunction were cross-sectionally related to cognitive decrements (**chapter 4**).

Overall, the hypothesis was confirmed that microvascular disease elsewhere in the body is important to the brain, as reflected in consistent associations between microvascular damage and cognitive functioning. However, the association with cognitive functioning in all studies was rather weak. Thus, despite their relation with cognitive functioning, and despite their easy way of being measured, these biomarkers of systemic microvascular disease are of limited value as markers of reduced cognitive performance in the population.

Part II – Cerebral markers of microvascular disease and cognitive functioning

In the second part of this thesis, measures of microvascular disease in the brain were investigated in patients with aMCI or early AD. **Chapter 6** describes that multiple microbleeds were associated with disruptions of the microstructure and organization of the cerebral network as measured with diffusion tensor imaging (DTI), independent of other measures of small vessel disease or atrophy. In **chapter 7**, we used 7 Tesla MRI to investigate the presence of microbleeds in detail. We showed that the majority of patients with aMCI or early AD exhibit cerebral microbleeds. In **chapter 8, 7** Tesla MRI was used to image cortical microinfarcts. We found that numbers were not significantly increased in patients relative to controls, but microinfarcts did appear to be related to cerebral microbleed numbers and to other, larger, cortical infarcts.

We showed that cerebral microvascular lesions are associated with (end-

stage) dementia presence, but not so much with cognitive functioning in patients with AD. This implies that microbleeds and microinfarcts are primarily markers of presence of brain pathology underlying cognitive impairment, rather than of its severity. Novel MRI techniques such as DTI and 7 Tesla MRI open the door for many future studies investigating the vascular burden in AD.

Part III – Experimental neuropsychology

In the third part of this thesis, we investigated a cognitive function that is often affected in patients with aMCI or early AD, but has received less attention: spatial navigation. In **chapter 9** three fundamental aspects of spatial navigation were studied, namely scene recognition, route continuation, and route ordering. Patients performed worse than controls, and patients with aMCI were at an intermediate level between controls and patients with AD. Because of the clinical relevance of spatial navigation deficits, we argue that assessment of navigation abilities in older populations should be part of standard clinical practice.

Conclusion

Microvascular disease is important with regard to cognition. An association between markers of microvascular disease and cognitive functioning is present in people without cognitive complaints, although this association is rather weak. Therefore, markers at the population level are not suitable for detection of individuals at risk for cognitive decline. In patients with aMCI or early AD, cerebral markers of microvascular disease are prevalent and are related to other vascular brain damage, but the association with cognitive functioning and clinical variables needs further study.

NEDERLANDSE SAMENVATTING

Cognitieve stoornissen en dementie vormen een belangrijk probleem voor de volksgezondheid en een aanzienlijk deel van de oudere bevolking wordt erdoor getroffen. Vaatschade in de hersenen wordt in toenemende mate gezien als een van de voornaamste oorzaken van cognitieve achteruitgang bij veroudering en dementie. Een andere oorzaak van cognitief verval wordt gevormd door neurodegeneratieve processen, die leiden tot pathologieën zoals we zien bij de ziekte van Alzheimer. Er is een groeiende hoeveelheid bewijs voor een cruciale rol van vaatschade bij de ziekte van Alzheimer. Ten eerste dragen risicofactoren voor vaatschade bij aan de ontwikkeling van de ziekte van Alzheimer. Daarnaast wordt bij een meerderheid van de ouderen bij autopsie een gemengd profiel gevonden van vaatschade en Alzheimer-pathologie. Deze bevindingen hebben geleid tot een nieuwe kijk op de oorzaken van cognitieve achteruitgang en dementie. Men gaat nu uit van een spectrum, dat loopt van patiënten met enkel vaatschade tot patiënten met enkel schade van het type van de ziekte van Alzheimer, en in het midden een grote groep patiënten met een gemengd beeld van beide typen schade. Het is daarom van belang dat wetenschappelijk onderzoek zich richt op de rol van vaatschade bij het ontstaan van cognitieve stoornissen, op zichzelf en in interactie met de ziekte van Alzheimer.

Vaatschade kan zich afspelen in zowel de grote als de kleine bloedvaten, en kan in beide gevallen de hersenen treffen. De kleine vaten zijn van fundamenteel belang voor het functioneren van de hersenen, en microvasculaire schade is dan ook van belang voor het gehele scala aan cognitief functioneren, van subtiele tekorten behorend bij normale veroudering, tot “mild cognitive impairment” (MCI; een overgangsstadium tussen normale cognitie en dementie) en ernstige dementie. Het onderzoek dat beschreven is in dit proefschrift, richt zich op de relatie tussen maten van microvasculaire schade (“biomarkers” of “markers”) en cognitief functioneren. Dit is onderzocht in de algemene oudere bevolking, waarbij gebruik gemaakt is van markers van microvasculaire schade in het lichaam, buiten de hersenen (deel I). Daarnaast zijn markers van microvasculaire schade in de hersenen onderzocht in patiënten met amnestische MCI (aMCI; MCI inclusief een geheugenstoornis, wat wordt gezien als een overgangsstadium tussen normale cognitie en de ziekte van Alzheimer) of vroege ziekte van

Alzheimer, waarbij gebruik gemaakt is van nieuwe "magnetic resonance imaging" (MRI) technieken (deel II). Ten slotte is een cognitieve functie onderzocht die vaak verminderd is in patiënten met aMCI of ziekte van Alzheimer (deel III).

Deel I – Systemische markers van microvasculaire schade en cognitief functioneren

In het eerste deel van dit proefschrift werden maten voor microvasculaire schade in delen van het lichaam buiten de hersenen onderzocht, en de relatie met cognitief functioneren. In **hoofdstuk 2** is een overzicht gegeven van de literatuur over kleine bloedvaten in het netvlies (retina) in dit verband. Retinale microvasculaire veranderingen waren gerelateerd aan dementie, aan milde cognitieve tekorten in mensen zonder dementie en aan afwijkingen op hersenscans. Sommige studies in het overzicht vonden geen significant verband tussen aanwezigheid van retinopathie en cognitief functioneren, wat ook het geval was in onze eigen studie in zelfstandig wonende ouderen zonder ernstige cognitieve problemen (**hoofdstuk 5**). In hetzelfde studiecohort werden echter wel associaties gevonden tussen andere systemische markers van microvasculaire schade en cognitief functioneren. **Hoofdstuk 3** beschrijft dat albuminurie, een maat voor microvasculaire schade aan de nieren, gerelateerd was aan milde cognitieve tekorten 5-7 jaar later. Bovendien waren biomarkers voor inflammatie en endotheeldysfunctie in het serum, cross-sectioneel gerelateerd aan cognitieve beperkingen (**hoofdstuk 4**).

Al met al werd de hypothese bevestigd dat microvasculaire schade elders in het lichaam van belang is voor de hersenen, aangezien er consistente associaties gevonden worden met cognitief functioneren. Deze associatie was echter in alle studies vrij zwak. Dus, hoewel er een relatie is met cognitief functioneren en hoewel deze markers eenvoudig gemeten kunnen worden, hebben deze systemische biomarkers van microvasculaire schade slechts beperkte waarde als marker voor verminderd cognitief functioneren in de algemene populatie.

Deel II – Cerebrale markers van microvasculaire schade en cognitief functioneren

In het tweede deel van dit proefschrift werden maten van microvasculaire schade in de hersenen onderzocht in patiënten met aMCI of vroege ziekte

van Alzheimer. **Hoofdstuk 6** beschrijft dat het hebben van meerdere microbloedingen was geassocieerd met verstoringen in de microstructuur en de organisatie van het cerebrale netwerk, gemeten met "diffusion tensor imaging" (DTI) MRI, onafhankelijk van andere maten van hersenschade. In **hoofdstuk 7** werden 7 Tesla MRI hersenscans gebruikt om de aanwezigheid van microbloedingen gedetailleerd te onderzoeken. We lieten zien dat de meerderheid van patiënten met aMCI of vroege ziekte van Alzheimer cerebrale microbloedingen vertonen. In **hoofdstuk 8** werden 7 Tesla MRI-scans gebruikt om corticale microinfarcten af te beelden. Deze bleken niet vaker voor te komen in patiënten dan in controleproefpersonen. Er waren echter wel aanwijzingen voor een relatie tussen microinfarcten en aantallen microbloedingen en andere, grote, corticale infarcten.

We hebben laten zien dat cerebrale microvasculaire laesies geassocieerd zijn met de aanwezigheid van dementie, maar niet zozeer met cognitief functioneren binnen patiënten met de ziekte van Alzheimer. Dit suggereert dat microbloedingen en microinfarcten in de eerste plaats markers zijn voor de aanwezigheid van hersenpathologie die ten grondslag ligt aan cognitieve stoornissen, maar in mindere mate voor de ernst van deze pathologie. Nieuwe MRI-technieken zoals DTI en 7 Tesla MRI bieden perspectieven voor toekomstig onderzoek naar de rol van vaatschade bij de ziekte van Alzheimer.

Deel III – Experimentele neuropsychologie

In het derde deel van dit proefschrift werd een cognitieve functie onderzocht die vaak aangedaan is in patiënten met aMCI of vroege ziekte van Alzheimer, maar waar tot nu toe weinig aandacht voor is geweest: spatiële navigatie. In **hoofdstuk 9** werden drie fundamentele aspecten van spatiële navigatie bestudeerd, namelijk het herkennen van de omgeving, het vervolgen van een route en het reproduceren van de volgorde van een route. Op alle aspecten presteerden patiënten slechter dan controleproefpersonen en lag het niveau van patiënten met aMCI tussen dat van de patiënten met vroege ziekte van Alzheimer en de controleproefpersonen in. Vanwege de klinische relevantie van problemen in het vermogen te navigeren, pleiten wij ervoor dat deze functie vaker in kaart wordt gebracht in oudere populaties.

Conclusie

Microvasculaire schade is van belang met betrekking tot cognitief functioneren. Er is een verband tussen markers van microvasculaire schade en cognitie in mensen zonder cognitieve klachten, ook al is dit verband vrij zwak. Om die reden zijn markers op het niveau van de bevolking niet geschikt om mensen met verhoogd risico op cognitieve achteruitgang te identificeren. In patiënten met aMCI of vroege ziekte van Alzheimer zijn cerebrale markers voor microvasculaire schade aanwezig en deze zijn gerelateerd aan andere vormen van vaatschade in de hersenen, maar het verband met cognitief functioneren en klinische variabelen behoeft nader onderzoek.

LIST OF PUBLICATIONS

SM Heringa, E van den Berg, JM Dekker, G Nijpels, RPC Kessels, LJ Kappelle, CDA Stehouwer, GJ Biessels. Albuminuria and cognitive functioning in an older population: The Hoorn Study. *Dementia and Geriatric Cognitive Disorders* 2011;32:182–187.

SM Heringa, WH Bouvy, E van den Berg, AC Moll, LJ Kappelle, GJ Biessels. Associations between retinal microvascular changes and dementia, cognitive functioning, and brain imaging abnormalities: a systematic review. *Journal of Cerebral Blood Flow and Metabolism* 2013;33:983-95.

SM Heringa, YD Reijmer, A Leemans, HL Koek, E van den Berg, LJ Kappelle, GJ Biessels. Microbleeds, cerebral white matter network disruptions and cognitive functioning in patients with early Alzheimer's disease. *Journal of Alzheimer's Disease* 2014;38:211-21.

SM Heringa, E van den Berg, G Nijpels, CDA Stehouwer, CG Schalkwijk, T Teerlink, PG Scheffer, K van den Hurk, YD Reijmer, LJ Kappelle, JM Dekker, GJ Biessels. Markers of low-grade inflammation and endothelial dysfunction are related to reduced information processing speed and executive functioning in an older population – the Hoorn Study. *Psychoneuroendocrinology* Psychoneuroendocrinology 2014;40:108-118.

SM Heringa, I Walraven, AC Moll, E van den Berg, JM Dekker, G Nijpels, LJ Kappelle, CDA Stehouwer, GJ Biessels. Retinal vascular changes and cognitive functioning in an older population – The Hoorn Study. Accepted for publication as a letter to the editor in: *Journal of the American Geriatrics Association*.

M Brundel, **SM Heringa**, J de Bresser, HL Koek, JJ Zwanenburg, LJ Kappelle, PR Luijten, GJ Biessels. High Prevalence of Cerebral Microbleeds at 7 Tesla MRI in Patients with Early Alzheimer's Disease. *Journal of Alzheimer's Disease* 2012;31:259-63.

SJ van Veluw, **SM Heringa**, HJ Kuijf, HL Koek, PR Luijten, GJ Biessels. Cerebral cortical microinfarcts at 7 Tesla MRI in patients with early Alzheimer's Disease. *Journal of Alzheimer's Disease* 2013, in press.

YD Reijmer, A Leemans, **SM Heringa**, I Wielaard, B Jeurissen, HL Koek, GJ Biessels. Improved sensitivity to cerebral white matter abnormalities

in Alzheimer's disease with spherical deconvolution based tractography. *PLoS One* 2012;7(8):e44074.

YD Reijmer, A Leemans, K Caeyenberghs, **SM Heringa**, HL Koek, GJ Biessels. Disruption of cerebral networks and cognitive impairment in Alzheimer's disease. *Neurology* 2013;80:1370-7.

HJ Kuijf, M Brundel, J de Bresser, SJ van Veluw, **SM Heringa**, MA Viergever, GJ Biessels, KL Vincken. Semi-Automated Detection of Cerebral Microbleeds on 3.0 T MR Images. *PLoS One*. 2013 21;8:e66610.

SM Heringa, E van den Berg, HL Koek, LJ Kappelle, GJ Biessels, IJM van der Ham. Spatial navigation in healthy older people and patients with amnestic mild cognitive impairment or Alzheimer's disease. In preparation.

THESES OF THE UTRECHT VASCULAR COGNITIVE IMPAIRMENT STUDY GROUP

1. SM Manschot, 2006. Diabetic encephalopathy: A cerebrovascular disorder? Utrecht University, Utrecht, the Netherlands.
2. AMA Brands, 2007. Diabetes and the brain: Cognitive performance in type 1 and type 2 diabetes mellitus. Utrecht University, Utrecht, the Netherlands.
3. E van den Berg, 2009. Type 2 diabetes and cognition: Neuropsychological sequelae of vascular risk factors in the ageing brain. Utrecht University, Utrecht, the Netherlands.
4. J de Bresser, 2011. MRI-based quantification of brain damage in cerebrovascular disorders. Utrecht University, Utrecht, the Netherlands.
5. YD Reijmer, 2012. Vascular Cognitive Impairment: Risk factors and brain MRI correlates. Utrecht University, Utrecht, the Netherlands.
6. HJ Kuijf, 2013. Image processing techniques for quantification and assessment of brain MRI. Utrecht University, Utrecht, the Netherlands.
7. SM Heringa, 2014. Cognitive functioning and microvascular disease. Utrecht University, Utrecht, the Netherlands.

DANKWOORD

De afgelopen jaren heb ik met veel plezier in het UMC Utrecht aan dit onderzoek gewerkt en heb ik mijzelf kunnen ontwikkelen. De mensen die dit mogelijk gemaakt hebben, wil ik graag bedanken.

In de eerste plaats bedank ik de patiënten, hun verzorgers en de gezonde proefpersonen, voor de tijd en moeite die zij hebben genomen om mee te doen aan de studies.

Professor dr. G.J. Biessels, beste Geert Jan, jij bent veruit de belangrijkste persoon geweest bij de totstandkoming van dit boekje. Ik heb mezelf altijd gelukkig geprezen met jou als begeleider en heb grote bewondering voor je snelle denkkracht. Ik ben er trots op dat ik deel mocht uitmaken van jouw bloeiende VCI-groep: een van je talenten is het bij elkaar brengen van slimme en sympathieke mensen en goed voor ze te zorgen. Dank voor alles wat je me geleerd hebt. Het is een eer om de eerste promovenda te zijn aan wie jij de bul uitreikt.

Professor dr. L.J. Kappelle, beste Jaap, je positiviteit, bemoedigende woorden en treffende opmerkingen bij artikelen zijn van grote waarde voor mij geweest, bedankt daarvoor.

Dr. E. van den Berg, beste Esther, dank je wel voor je betrokken begeleiding. Je hebt me op zo veel vlakken geholpen en adviezen gegeven: dat geldt niet alleen voor het schrijven, maar ook voor het leven als aio en neuropsycholoog. Je bent een voorbeeld voor me, zoals je wetenschappelijk en klinisch neuropsychologisch onderzoek in je werk met elkaar verbindt. Bedankt dat je altijd in me geloofd hebt.

De leden van de beoordelingscommissie, Prof. dr. P.R. Luijten, Prof. dr. A. Algra, Prof. dr. A Postma, Prof. dr. F.L.J. Visseren en Prof. dr. J.M. Dekker, dank ik voor hun bereidheid dit manuscript te lezen en te beoordelen.

Voor het project Parelsnoer Neurodegeneratieve Ziekten heb ik met veel mensen mogen samenwerken.

Ik wil iedereen op de geriatrie bedanken. Jullie goedlopende geheugenpoli is erg belangrijk voor ons, neurologie-onderzoekers. Dineke Koek, bedankt dat je de geriatrieafdeling onophoudelijk weet te enthousiasmeren voor Parelsnoer en dank voor je inhoudelijke interesse. Marianne Versloot, bedankt, ik heb vanaf het begin erg prettig met je samengewerkt en heb organisatorisch veel van je opgestoken.

Dank aan Els Vriens, Richard Faaij, Marije Hamaker en Machiel Pleizier in het Diakonessenhuis in Zeist, voor de impuls die jullie aan het project hebben gegeven.

De stagiaires die onmisbaar waren voor het testen en begeleiden van de patiënten, Sanne, Irene, Ilse, Kiekie, Bodi, Whitney en Jonne: bedankt dat jullie allemaal zo enthousiast en leergierig waren.

Joyce de Wit en Kim Blom, het is nu aan jullie. Ik weet zeker dat de patiënten en de organisatie bij jullie in goede handen zijn. Bedankt voor de fijne overdracht.

Dank aan de projectleiders van de Hoorn Studie voor het gebruiken van deze mooie dataset. Jacqueline Dekker in het bijzonder, en ook Giel Nijpels, Coen Stehouwer en alle coauteurs, wil ik bovendien bedanken voor hun snelle, kritische en motiverende commentaren. Annette Moll en Iris Walraven, dank voor al het werk dat jullie gedaan hebben voor de retina-stukken.

Alexander Leemans, ik ben blij dat ik een DTI-artikel heb mogen schrijven, bedankt voor je hulp daarbij.

Ineke van der Ham, bedankt dat we het leuke navigatieproject samen met jou konden doen en dat je me daarmee een kijkje hebt gegeven in het experimenteel onderzoek.

Mijn tijd in het UMC zal ik me blijven herinneren als een zonnige periode, waarvoor mijn geweldige collega's een hoop credits verdienen.

Zowel met de VCI-groep als met Kamertje 1 heb ik het enorm getroffen. Lieve Yael, Susanne, Linda, Manon, Willem, Lieza, Laura, Minke, Rachel, Janneke, Marcel, Onno, Joyce, Kim, Jeroen, Eduard, Monique, Merel,

Casper, Raoul en overige VCI'ers: ontzettend bedankt! De kracht van het samen promoveren is ongekend en levert naast publicaties bovenal steun en gezelligheid op. Menig aio zal de vreugde van appelbollen, congressen, etentjes en borrels herkennen. Met jullie heb ik bovendien mogen genieten van de eierkoker, seizoensversieringen, tjap tjoy, corona's en cactuswaterschema's. En wat een feest dat er altijd wel een reden was voor een pouletje.

Een aparte vermelding is er natuurlijk voor Yael, ik had je er heel graag bij gehad op mijn promotie. Dank voor al je harten onder de riem.

De neuropsychologen: Martine, Carla, Haike, Irene en Esther, bedankt dat ik er altijd een beetje bij mocht blijven horen. Bij jullie ben ik mijn UMC-tijd begonnen en met jullie deel ik de liefde voor de kliniek. Martine, bedankt voor je vertrouwen in mij als neuropsycholoog en voor je aanstekelijke bevlogenheid voor het vak.

Broodnodige afleiding was er vaak tijdens de lunch, ook met minder directe collega's: bedankt Janneke, Judith, Linda en Greetje.

Dan zijn er buiten het UMC veel lieve mensen die ik wil bedanken voor de afgelopen jaren.

Tutti Frutti zorgt al tien jaar voor dinsdagse gezelligheid, vakanties en veel meer. Annemarie, Daniëlle, Duveke, Floor, Froukje, Heleen, Kelly, Laura, Lea, Manja, Marije en Marlies, bedankt, toppertjes!

Het USKO heeft mij vanaf het allereerste begin van mijn studententijd veel gebracht. Nu nog pluk ik er de vruchten van, doordat mensen als Jef, Froukje, Renée, Renate, Ernestine, Duveke, Anneloes, Katrien, Welmoet, Sanne, Laura, Janneke, Yvon, David en Iris muziek in mijn leven brengen. Dank jullie wel!

Frankrijkvriendinnen, Laura, Carolien, Mirjam en Corianne, bedankt voor de weekendjes en goede gesprekken.

Irma, bedankt dat je zo'n trouwe vriendin bent. Ik vind het altijd erg leuk om het met jou over het ziekenhuisleven te hebben. Sanne, bedankt voor de vele skype-uurtjes. Kom je gauw terug? Marieke, engel, bedankt voor je vriendschap. Met jou is het heerlijk herinneringen ophalen.

 Kuin, wat heb je een prachtig schilderij gemaakt, precies zoals ik het wilde.

Lieve paranimfen, Froukje en Jouke. Ik ben blij dat juist jullie achter me zullen staan.

Froukje, sinds de 1e Daalsedijk ben je een dierbare vriendin. Dank voor al je steun.

Jouke, bro, dank voor onze sterke verbondenheid. Wat een geluk dat er iemand is met wie ik zo veel geschiedenis en zo exact dezelfde humor kan delen.

Kaarina, bedankt voor je grootse warmte. Alle goeds voor jou, Jouke en de kleine Matthias.

Bram en Reina, dank jullie wel dat jullie mijn lieve ouders zijn. Ik weet dat jullie trots op me zijn en dat maakt me gelukkig.

Lieve Onno, dank je wel dat je zo onverbeterlijk lief voor me bent. Je bent absoluut het mooiste dat onderzoek doen me heeft opgeleverd.

CURRICULUM VITAE

Sophie Heringa was born May 21st, 1984 in Usselo (Enschede), the Netherlands. She attended secondary school at the Stedelijk Gymnasium Breda, from which she graduated in 2002. In 2002-2003, she performed voluntary work in Geneva, Switzerland and Paris, France. In September 2003, she started her studies in psychology at Utrecht University. As a part of her bachelor's degree, she studied for one semester at Istanbul Bilgi University, Turkey. In 2007 she started the master Neuropsychology at Utrecht University. Her enthusiasm for both clinical and scientific aspects of neuropsychology was stimulated during a clinical internship under supervision of dr. M.J.E. van Zandvoort at the Neurology department at University Medical Center Utrecht (UMCU). After graduating in January 2009, she started as a research assistant at the same department. Between 2009 and 2012, she coordinated the Parelsnoer Neurodegenerative Diseases project at the UMCU. This work led to a PhD project resulting in this thesis, under supervision of prof. dr. G.J. Biessels, prof. dr. L.J. Kappelle, and dr. E. van den Berg. Sophie will now focus on a career as a clinical neuropsychologist.