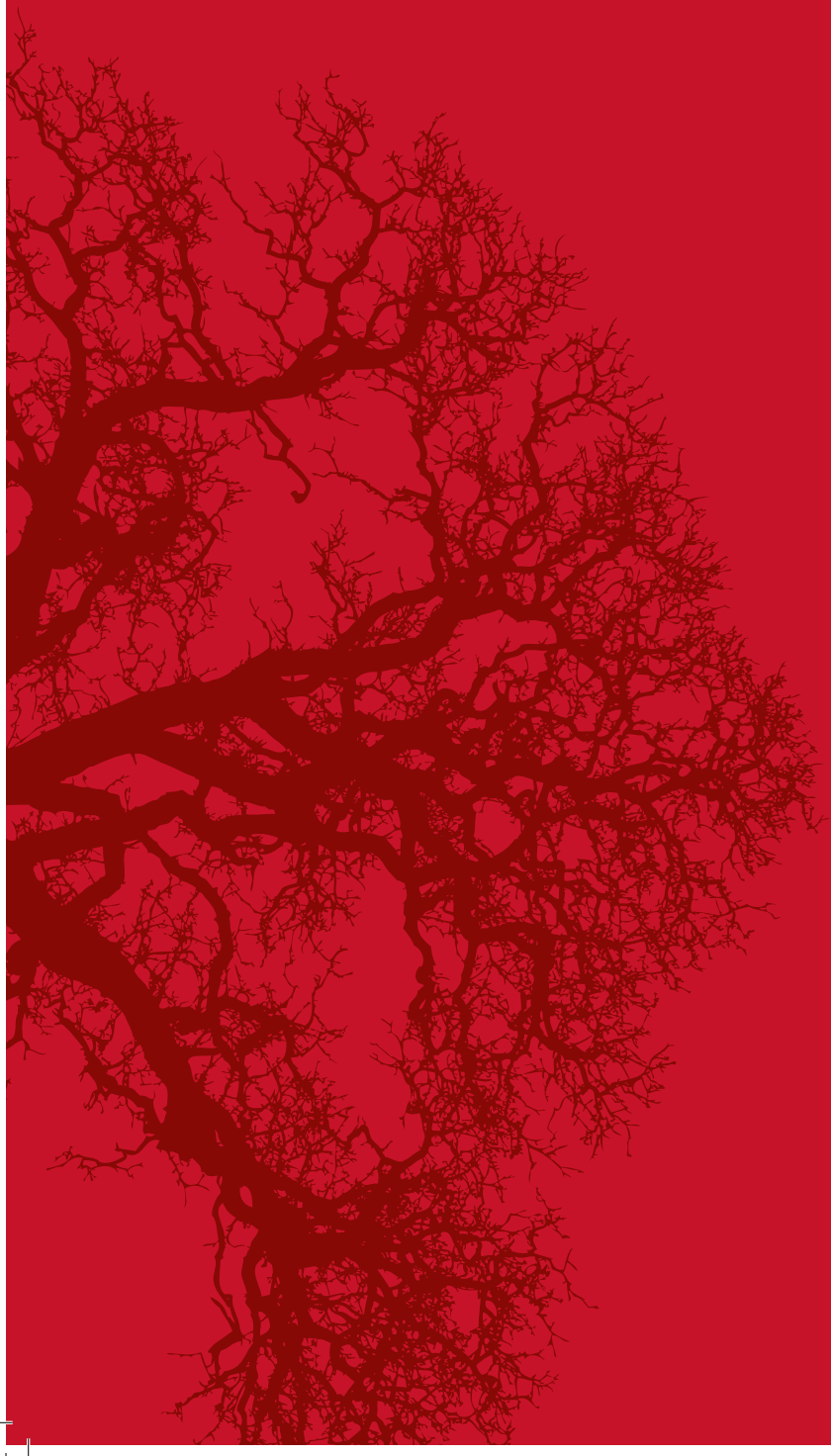


Cover and lay-out: Teuntje van de Wouw
Printed by: Ipskamp Drukkers B.V.
ISBN: 978-90-393-6187-0

DIABETES AND MICROVASCULAR DISEASE IN VASCULAR COGNITIVE IMPAIRMENT

DIABETES EN MICROVASCULAIRE SCHADE
BIJ VASCULAIRE COGNITIEVE BEPERKINGEN

(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 2 september 2014 des middags om 4.15 uur

door

Manon Brundel

geboren 15 februari 1984 te 's-Gravenhage

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

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

Financial support was generously provided by Philips Healthcare Nederland, Chipsoft, Novo Nordisk BV, the Internationale Stichting Alzheimer Onderzoek, Nutricia Advanced Medical Nutrition, and Boehringer Ingelheim.

CONTENTS

1 GENERAL INTRODUCTION

1

Vascular cognitive impairment

Dementia is a syndrome characterized by an acquired impairment of cognitive function in at least two domains, interfering with daily activities.[1] It is frequently diagnosed, with a prevalence of 5-7% after age 60. Worldwide, it has been estimated that more than 35 million people have dementia, a number which is expected to double every 20 years due to increasing life expectancy.[2] Therefore, in our ageing society, cognitive impairment and dementia are a major public health problem.
The most common form of dementia is Alzheimer's Disease (AD). AD typically has a gradual onset and presents predominantly with progressive memory impairment, along with other cognitive deficits.[3] From a neuropathological point of view typical changes associated with AD include protein deposits, forming amyloid-beta plaques, and neurofibrillary tangles.

The second most prevalent form of dementia is Vascular Dementia (VaD). Traditionally, VaD has been defined as dementia caused by cerebrovascular disease, characterized by either a clinical stroke, or evidence of significant cerebrovascular disease on brain imaging. These brain imaging abnormalities include either multiple infarcts or a single strategically located infarct, but can also consist of extensive periventricular white matter lesions. According to the classical definition of vascular dementia cognitive dysfunction should become manifest shortly after a stroke is diagnosed, or should show a fluctuating, stepwise progression.[4] Obviously, this definition does not capture the wide variety of cognitive deficits related to cerebrovascular damage. Therefore, the term Vascular Cognitive Impairment (VCI) has been introduced, to refer to the entire spectrum of cognitive disorders, ranging from mild cognitive impairment to clear dementia, associated with and presumed to be caused by all forms of cerebrovascular disease.[5] With this definition, also more insidious cognitive and structural brain changes are recognised. Moreover, vascular causes are now pointed out as a contributing factor, instead of a direct cause, in the development of cognitive decline.

VCI also comprises different pathophysiological processes. One important underlying process is small vessel disease (SVD). It is described as all the pathological processes that affect the small vessels of the brain, including small perforating arteries, arterioles, capillaries, and venules.[6] The consequences of the pathological changes of these small vessels on the brain parenchyma are also referred to as SVD. Brain imaging, mainly magnetic resonance imaging (MRI), plays an important role in the detection of SVD. Brain MRI features of SVD are lacunar infarcts, white matter hyperintensities (WMH), prominent perivascular spaces, cerebral microbleeds, and global brain atrophy (see figure 1).[7] These features also constitute the most common MRI markers of VCI.

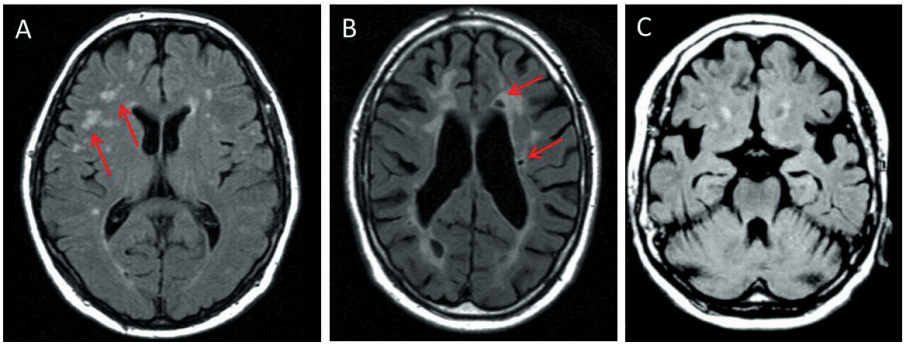


Figure 1 MRI markers of small vessel disease and Vascular Cognitive Impairment: white matter hyperintensities (A), lacunar infarcts (B) and global brain atrophy (C)

Historically, AD en VaD have been considered as separate entities. The past decades however, this tight categorical view is being re-evaluated. Importantly, Alzheimer-type pathology and vascular brain damage mostly co-occur at autopsy.[8] Moreover, cerebral amyloid angiopathy (deposits of the protein amyloid in blood vessels) has been acknowledged as an important marker of AD, but also of VaD. Furthermore, vascular risk factors, such as hypertension, diabetes, hypercholesterolemia and obesity, increase the risk both for cerebrovascular disease and for a clinical diagnosis of AD. Therefore, it seems to be more adequate to consider pure AD and pure VaD pathology as ends of a spectrum, with most patients exhibiting a combination of both pathologies. VCI refers to the contribution of vascular brain pathology across this whole spectrum.

Vascular risk factors are modifiable. Given the close association between vascular risk factors and dementia, treatment of these risk factors may give an opportunity to prevent cognitive decline. For the development of future therapeutic interventions, further insight in the natural history and underlying mechanisms of VCI is required.

This thesis focuses on two specific aspects of VCI:

- To address type 2 diabetes mellitus as a risk factor for cognitive decline in the context of vascular brain damage.
- To explore cerebral microvascular lesions as etiological and prognostic marker of VCI.

In both topics, MR imaging techniques play a key role. The following paragraphs will give a short introduction to the different research questions addressed in both parts of the thesis.

Type 2 diabetes mellitus as a risk factor for VCI

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder, that affects around 382 million people worldwide,[9] particularly patients of 60 years and older.[10] It is characterized by a relative insulin deficiency and chronic hyperglycaemia. T2DM is associated with mild to moderate cognitive decrements, particularly in the cognitive domains attention and executive functioning, information processing speed, and memory, and with a twice increased risk of dementia.[11,12] The aetiology of T2DM associated cognitive dysfunction is still incompletely known. However, vascular brain disease is likely to play a role, since T2DM predisposes to macro- and microvascular disease, both in the brain and elsewhere in the body.[13]

Brain imaging gives important information about processes underlying the observed cognitive changes in T2DM. Imaging studies have indeed established that T2DM is accompanied by certain brain abnormalities: brain atrophy and lacunar infarcts appear to be more common in patients with T2DM than in controls.[14-16] The association between T2DM and WMHs is not completely clear.[14,17,18] It is also uncertain if brain atrophy in T2DM is global, or if there are specific vulnerable areas. For example, the medial temporal lobe has been suggested to be particularly affected.[19] Other open questions with respect to brain imaging abnormalities in T2DM relate to whether, besides structural changes, also functional changes, e.g. altered cerebral haemodynamics, play a role, and whether microvascular lesions are more frequent in patients with T2DM and if they are a contributor to T2DM associated cognitive impairment.

These issues will be addressed in the first part of this thesis, to understand better the underlying causes of T2DM associated cognitive dysfunction.

Novel imaging markers of VCI: microinfarcts and microbleeds

Conventional imaging markers of SVD (i.e. lacunar infarcts, WMH, brain atrophy) do not capture the full burden of cerebral microvascular damage. Autopsy studies point to the relevance of microvascular lesions, in particular microinfarcts, in cognitive decline and dementia. Neuropathologically, microinfarcts are focal lesions attributed to ischemia, usually defined as 'not visible with the naked eye'. They are a common finding at autopsy, in the general population, and appear to be more prevalent in patients with cerebrovascular disease, vascular dementia, and Alzheimer's disease.[20-22] However, evidence on these associations originates from post-mortem data and therefore the relation with age, risk factors, and disease progression is largely unknown. This information however is extremely important

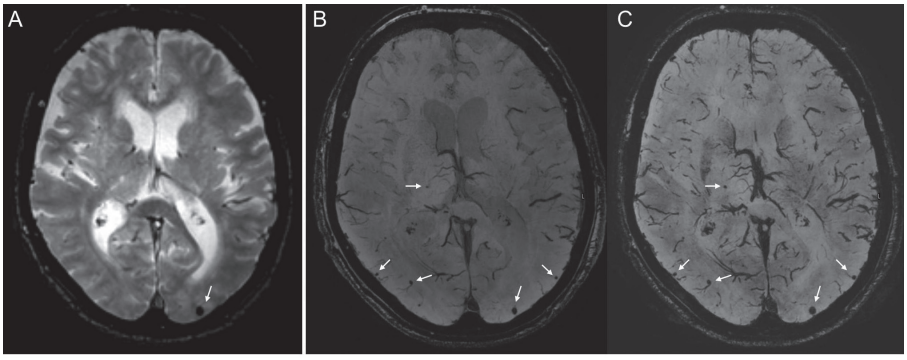


Figure 2 Increased visibility of cerebral microbleeds with increasing field strength: an example of a single slice T2*-weighted MR image at 3 Tesla MRI (A), and 7 Tesla MRI (first echo (B) and second echo (C)), showing four extra microbleeds at 7 Tesla MRI (arrows).

to understand the etiology and to finally develop therapeutic interventions in VCI. Higher MR field strength and new imaging techniques now probably make it possible to visualize microinfarcts *in vivo*.

Ultra-high field strength (7 Tesla) MRI also greatly enhance the detection of another type of microvascular lesions: cerebral microbleeds. These lesions are visible as small black dots on T2*-weighted MR sequences.[7] Histopathological studies indicate that microbleeds reflect hemosiderin-laden macrophages in perivascular tissue consistent with vascular leakage of blood cells.[23] These hemosiderin deposits are paramagnetic and induce a susceptibility effect on T2*-weighted MR imaging, which appears as a signal void. 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.[4] Moreover, due to the so-called blooming effect, the visualized size of the signal void increases with field strength and echo time. Using different echo times at 7 Tesla MRI increases detectability of microbleeds (see figure 2). Previous studies at 7 Tesla MRI showed that even small microbleeds were visible and that the number of detected microbleeds was three times higher compared to 1.5 Tesla MRI.[25,26]

Microbleeds have attracted attention since it became clear that they are related to vascular risk factors,[27] other MRI markers of small vessel disease,[27] and stroke.[28] Furthermore, they have been associated with cognitive impairment in the general population.[29] In stroke populations however, this relationship with cognition is less clear.[30,31] Etiologically, it has been suggested that microbleeds in deep regions of the

brain are associated with small vessel disease and hypertension as a main risk factor, whereas lobar microbleeds are closely linked to AD and its presumed pathological correlate, amyloid pathology.[28,32] However, the prevalence of microbleeds on MRI in patients with AD is lower than that of amyloid angiopathy at autopsy. This raises the question whether this discrepancy could be due to insufficient detection of microbleeds *in vivo*. The possibility to detect more microbleeds with new imaging techniques, allows further studies about their occurrence in different populations, both healthy and diseased, and to investigate their role in brain functionality, i.e. cognitive performance. It might well be that associations with clinical outcomes change with a better detection of microbleeds.

The UMC Utrecht was one of the first medical centers in the Netherlands to acquire a 7 Tesla MRI scanner, which has given us the opportunity to study microvascular lesions in the brain in our research projects with unprecedented detail.

In part II of this thesis I will pursue a number of specific aspects of microvascular lesions.

With respect to microinfarcts, I first will review the data from neuropathological studies on microinfarcts, to form a frame of reference for future MRI studies. During my PhD program, we succeeded in visualizing microinfarcts at 7 Tesla MRI. Subsequently, I investigated the association between microinfarcts at 7 Tesla MRI and age, vascular risk factors (including T2DM), and other MRI markers of SVD.

Regarding microbleeds, I focused on the following aspects: with 7 Tesla MRI I have assessed the relation between microbleeds and age, vascular risk factors and other MRI markers of SVD, and I examined whether they occur more frequently in patients with T2DM. I studied the occurrence of microbleeds also in patients with early AD. Furthermore, I addressed the question if microbleeds are related to cognitive performance in patients with a TIA or ischemic stroke, both at regular and at 7 Tesla MR field strength.

Outline of the thesis

In **part I**, we investigated different brain imaging markers in T2DM and the relationship with cognitive performance.

Chapter 2 provides a review on brain imaging in T2DM. It addresses the relationship between T2DM and brain abnormalities assessed with different MR imaging techniques, both structural and functional.

In **chapter 3** we investigated regional cerebral cortical atrophy in patients with

T2DM and non-diabetic controls. Furthermore, determinants of cortical atrophy within the T2DM group were examined.
In **chapter 4**, we examined how cerebral haemodynamics (cerebral blood flow and cerebrovascular reactivity) are related to cognitive functioning and brain volumes, at baseline and after four years, in individuals with T2DM.
Finally, in **chapter 5** we compared the occurrence of cortical microinfarcts and cerebral microbleeds at 7 Tesla MRI between nondemented older individuals with T2DM and control participants, and investigated the relationship with cognitive performance.

In **part II**, we focus on cerebral microvascular lesions in different populations, and their relationship with cognitive performance. We explore new imaging techniques to study these lesions.
Chapter 6 provides a review of the literature on neuropathological studies of cerebral microinfarcts, as a frame of reference for further MRI studies.
In **chapter 7** we examined the occurrence of both microbleeds and microinfarcts at 7 Tesla MRI in a sample of persons without major neurological conditions, and investigated the relationship with age, vascular risk factors and other imaging markers of small vessel disease.
In **chapter 8**, we examined the prevalence of cerebral microbleeds at 7 Tesla MRI in patients with mild cognitive impairment or early stages of Alzheimer's Disease, compared to controls.
In **chapter 9**, we examined the relationship between microbleeds at conventional field strength in patients with a TIA or minor ischemic stroke, and global cognitive performance four years later.
In **chapter 10**, we used 7 Tesla MRI to further study the relationship between microbleeds and cognitive performance in detail, in patients with a TIA or ischemic stroke.

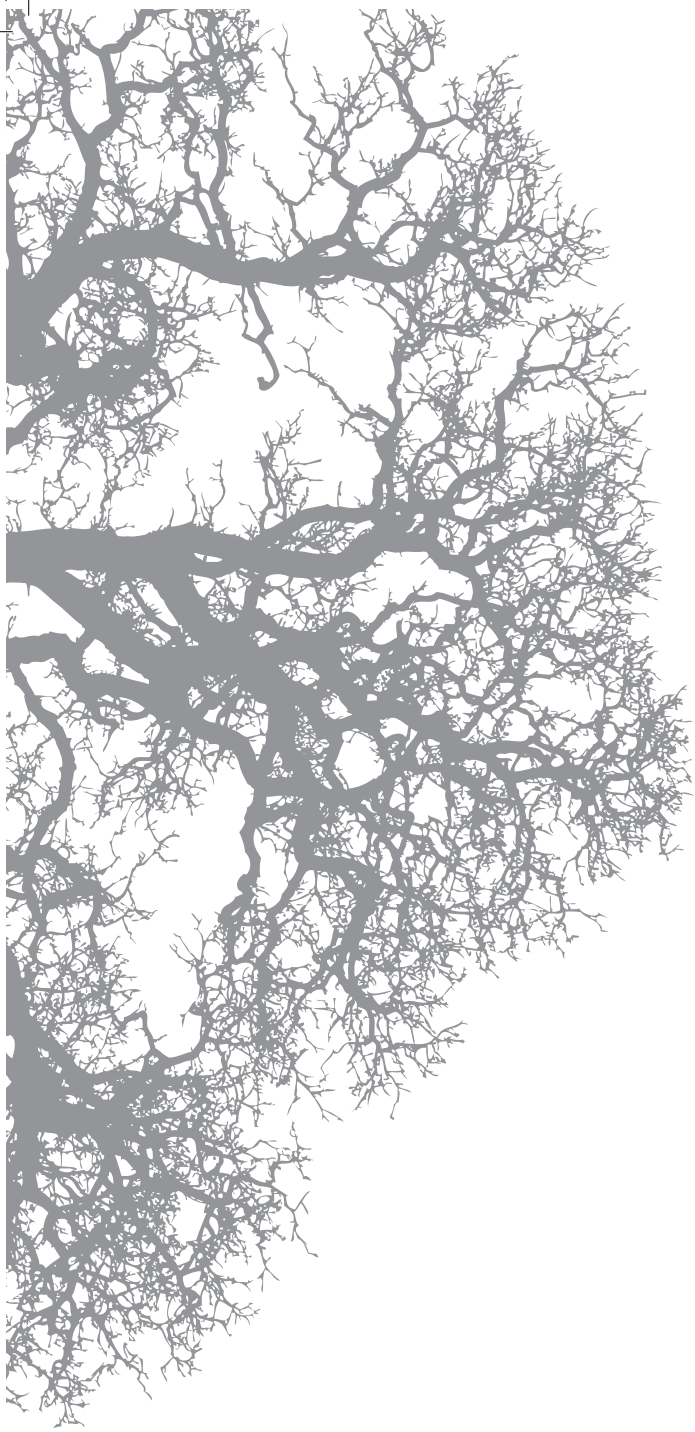
In **chapter 11** the results presented in this thesis are discussed as well as directions for future research and implications for clinical care.

REFERENCES

1. Ritchie K, Lovestone S. The dementias. *Lancet* 2002;360:1759–1766.
2. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement* 2013;9:63-75.
3. McKhann G, Knopman DS, Chertkow H, Hyman BT, Clifford RJ, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:263-269.
4. Roman GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Madeu JC, Garcia JH, Amaducci L, Orgogozo JM, Brun A, Hofman A. Vascular dementia; diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology* 1993;43:250-60.
5. O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, Bowler JV, Ballard C, DeCarli C, Gorelick PB, Rockwood K, Burns A, Gauthier S, DeKosky ST. Vascular Cognitive Impairment. *Lancet Neurol* 2003;2:89-98.
6. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical charachteristics to therapeutic challenges. *Lancet Neurol* 2010;9:689-701.
7. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, Lindley RI, O'Brien JT, Barkhof F, Benavente OR, Black SE, Brayne C, Breteler M, Chabriat H, Decarli C, de Leeuw FE, Doubal F, Duering M, Fox NC, Greenberg S, Hachinski V, Kilimann I, Mok V, Van Oostenbrugge R, Pantoni L, Speck O, Stephan BC, Teipel S, Viswanathan A, Werring D, Chen C, Smith C, van Buchem M, Norrving B, Gorelick PB, Dichgans M; STandards for ReportIng Vascular changes on nEuroimaging (STRIVE vi). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* 2013;12:822-838.
8. 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.
9. International Diabetes Federation, 2013. IDF Diabetes Atlas, sixth ed. International Diabetes Federation, Brussels, Belgium.
10. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* 2005;365:1333:1346.
11. Biessels GJ, Deary IJ, Ryan CM. Cognition and diabetes: a lifespan perspective. *Lancet Neurol* 2008;7:184-190.
12. Awad N, Gagnon M, Messier C. The relationship between impaired glucose tolerance, type 2 diabetes, and cognitive function. *J Clin Exp NEuropsychol*. 2004;26:1044-1080.

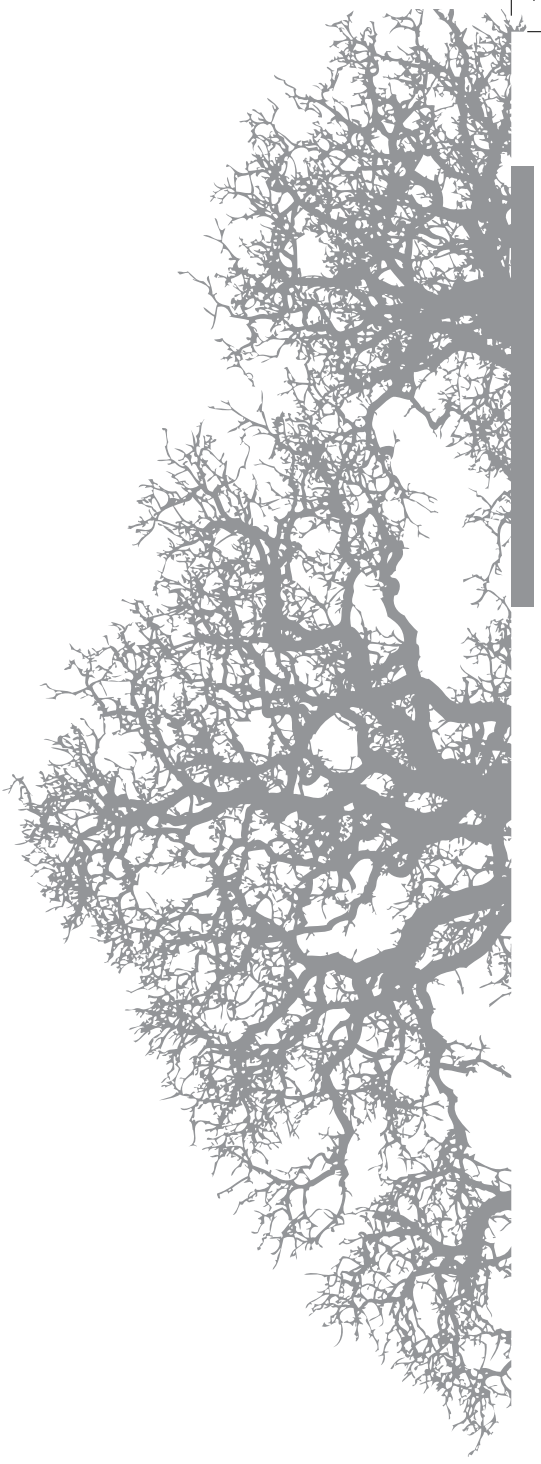
13. Luitse MJ, Biessels GJ, Rutten GE, Kappelle LJ. Diabetes, hyperglycaemia, and acute ischaemic stroke. *Lancet Neurol* 2012;11:261-271.
14. Jongen C, van der Grond J, Kappelle LJ, Biessels GJ, Viergever MA, Pluim JP. Automated measurement of brain and white matter lesion volume in type 2 diabetes mellitus. *Diabetologia* 2007;50:1509-1516.
15. Van Elderen SG, de Roos A, de Craen AJ, Westendorp RG, Blauw GJ, Jukema JW, Bollen EL, Middelkoop HA, van Buchem MA, van der Grond J. Progression of brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. *Neurology* 2010;14:997-1002.
16. Espeland MA, Bryan RN, Goveas JS, Robinson JG, Siddiqui MS, Liu S, Hogan PE, Casanova R, Coker LH, Yaffe K, Masaki K, Rossom R, Resnick SM. Influence of type 2 diabetes on brain volumes and changes in brain volumes: results from the Women's Health Initiative Magnetic Resonance Imaging studies. *Diabetes Care* 2013;36:90-97.
17. Schmidt R, Launer LJ, Nilsson LG, Pajak A, Sans S, Berger K, Breteler MM,de Ridder M, Dufouil C, Fuhrer R, Giampaoli S, Hofman A. Magnetic resonance imaging of the brain in diabetes: the Cardiovascular Determinants of Dementia (CASCADE) Study. *Diabetes* 2004;53:687-692.
18. Knopman DS, Mosley TH, Catellier DJ, Sharrett AR. Cardiovascular risk factors and cerebral atrophy in a middle-aged cohort. *Neurology* 2005;27:876-881.
19. Den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, et al. Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia* 2003;46:1604-1610.
20. Erkinjuntti T, Haltia M, Palo J, Sulkava R, Paetau A. Accuracy of the clinical diagnosis of vascular dementia: a prospective clinical and post-mortem neuropathological study. *J Neurol Neurosurg Psychiatry* 1988;51:1037-1044.
21. Arvanitakis Z, Leurgans SE, Barnes LL, Bennett DA, Schneider JA. Microinfarct pathology, dementia, and cognitive systems. *Stroke* 2011;42:722-727.
22. Esiri MM, Wilcock GK, Morris JH. Neuropathological assessment of the lesions of significance in vascular dementia. *J Neurol Neurosurg Psychiatry* 1997;63:749-753.
23. Shoamanesh A, Kwok CS, Benavente O. Cerebral microbleeds: histopathological correlation of neuroimaging. *Cerebrovasc Dis* 2011;32:528-534.
24. 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 Neurol* 2009;8:165-174.
25. Conijn MM, Geerlings MI, Biessels GJ, Takahara T, Witkamp TD, Zwanenburg JJ, Luijten PR, Hendrikse J. Cerebral microbleeds on MR imaging: comparison between 1.5 and 7T. *AJNR Am J Neuroradiol* 2011;32:1043-1049.

26. 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. *J Magn Reson Imaging* 2011;33:782-791.
27. Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Niessen WJ, Hofman A, Krestin GP, Breteler MMB. Prevalence and risk factors of cerebral microbleeds: The Rotterdam Scan Study. *Neurology* 2008;70:1208-1214.
28. Cordonnier C, Al-Shahi Salman R, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain* 2007;130:1988-2003.
29. 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. *Neurology* 2012;78:326-333.
30. Gregoire SM, Scheffler G, Jager 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* 2013;44:1267-1272.
31. Lawrence AJ, Patel B, Morris RG, MacKinnon AD, Rich PM, Barrick TR, Markus HS. Mechanisms of Cognitive Impairment In Cerebral Small Vessel Disease: Multimodal MRI results from the St George's Cognition and Neuroimaging in Stroke (SCANS) Study. *PloS One* 2013;8:e61014.
32. Cordonnier C, van der Flier WM. Brain microbleeds and Alzheimer's disease: innocent observation or key player? *Brain* 2011;134:335-344.



PART I

BRAIN IMAGING MARKERS IN TYPE 2 DIABETES



2

BRAIN IMAGING IN TYPE 2 DIABETES

M Brundel

LJ Kappelle

GJ Biessels

Published in: *Eur Neuropsychopharmacol* 2014 [Epub ahead of print]

ABSTRACT

Type 2 diabetes mellitus (T2DM) is associated with cognitive dysfunction and dementia. Brain imaging may provide important clues about underlying processes. This review focuses on the relationship between T2DM and brain abnormalities assessed with different imaging techniques: both structural and functional magnetic resonance imaging (MRI), including diffusion tensor imaging and magnetic resonance spectroscopy, as well as positron emission tomography and single-photon emission computed tomography.

Compared to people without diabetes, people with T2DM show slightly more global brain atrophy, which increases gradually over time compared with normal aging. Moreover, vascular lesions are seen more often, particularly lacunar infarcts. The association between T2DM and white matter hyperintensities and microbleeds is less clear. T2DM has been related to diminished cerebral blood flow and cerebrovascular reactivity, particularly in more advanced disease. Diffusion tensor imaging is a promising technique with respect to subtle white matter involvement. Thus, brain imaging studies show that T2DM is associated with both degenerative and vascular brain damage, which develops slowly over the course of many years. The challenge for future studies will be to further unravel the etiology of brain damage in T2DM, and to identify subgroups of patients that will develop distinct progressive brain damage and cognitive decline.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder, that affects around 382 million people worldwide, and prevalences are increasing.[1] It is characterized by reduced insulin sensitivity, followed by a compensatory increase in insulin secretion, and finally pancreas failure. T2DM and also pre-diabetic stages (with insulin resistance but normal glucose levels) are generally accompanied by a cluster of risk factors (dyslipidemia, hypertension, obesity), the so-called metabolic syndrome. These conditions predispose to atherosclerosis, cardiovascular disease and stroke.[2] Moreover, both T2DM and the metabolic syndrome can lead to microvascular complications, affecting the kidneys, eyes, peripheral nervous system and also the brain. Indeed, T2DM is associated with cognitive dysfunction and dementia.[3] Pathophysiological mechanisms for diabetes-related cognitive dysfunction are not entirely clear. Imaging can help us to detect brain abnormalities and to identify etiological factors. This review will address both structural and functional brain imaging studies in people with T2DM. Studies in people with pre-diabetic stages with impaired glucose tolerance (including the metabolic syndrome) will also be described. We focus primarily on studies using magnetic resonance imaging (MRI), but will also address radionuclide techniques. Computed tomography (CT) will be disregarded because MRI provides more detailed information on brain structure and function.

BRAIN ATROPHY AND VASCULAR LESIONS

Brain atrophy

Brain atrophy refers to brain volume loss not related to a specific macroscopic focal injury such as trauma or infarction.[4] It can be generalized or focal (affecting specific brain regions, e.g. the hippocampus), and can be further subdivided in subcortical atrophy (enlargement of the ventricles) and cortical atrophy (enlargement of the cortical sulci). Neuropathological correlates of atrophy are heterogeneous and can include not only neuronal loss, but also cortical thinning, subcortical vascular pathology and white matter rarefaction.[5] In normal aging, global brain atrophy becomes apparent particularly after the age of 70 years, with an average decline in total brain volume of 0.10-0.50% per year. [6,7] Brain atrophy, both globally and regionally, is associated with small vessel disease.[8,9] and worse cognitive performance.[10]

Cerebral atrophy can be assessed with ordinal rating scales and with CSF:brain ratios, e.g. the frontal interhemispheric fissure ratio and the Sylvian fissure ratio for cortical atrophy, and the bicaudate ratio and bifrontal ratio for subcortical atrophy.[11] For hippocampal atrophy, the Medial Temporal Atrophy (MTA) score is frequently used, which ranges from 0-3 (a scan from a subject is compared with a template).[12] Actual volumes of different brain regions, e.g. the hippocampus, can be determined by manually outlining of structures. More recently, automated segmentation methods have been developed.[13] They classify each voxel as gray matter, white matter or CSF, and quantify brain volumes unbiased and much more precisely. In cortical thickness measurements, the boundaries between gray and white matter and between gray matter and CSF are defined, allowing reconstruction of the cortical surface.

Brain atrophy in T2DM

Several studies investigated the relationship between diabetes and brain atrophy, some only cortical or subcortical atrophy, some both. The majority involved cohorts of community-dwelling people;[14-18] or case-control designs;[19-24] some described clinic based cohorts.[25,26] Most studies excluded people with known dementia and took possible confounding effects of other vascular risk factors into account.

In middle-aged subjects, T2DM has been consistently associated with global brain atrophy[19,22] or only subcortical atrophy,[15,23,27] both with global visual rating scales19,[27] as well as with automated volumetric methods.[22,23] In older subjects with T2DM (70-85 years of age), smaller total brain volumes,[14,17,25,26] smaller white matter volumes,[17,23] smaller gray matter volumes[14,17,26] and larger CSF volumes[22,23] have been reported. Reductions in mean total brain volume of 0.5-2.0% relative to controls have been reported, comparable with 2-5 years of normal aging. Of note, also in obese adolescents, T2DM was associated with smaller total brain volumes[28] and presence of the metabolic syndrome with larger brain CSF volumes.[29] Even in non-diabetic subjects, higher HbA1c and higher fasting glucose levels were associated with global brain atrophy[30] or subcortical atrophy.[15,17]

Recently, data from longitudinal studies on the progression of atrophy in patients with T2DM have become available. Over the course of three to six years, a slightly increased brain atrophy rate [25,26,30] or a greater increase in lateral ventricular volume[20,31] was reported in middle-aged to older aged subjects with higher HbA1c values or in subjects with T2DM compared with controls. Reported reductions in brain volume are small (0.1-1.5%, 1-3 times the atrophy rate of normal aging).

Hypertension has been found as a possible determinants of cerebral atrophy in

patients with T2DM,[18,20] although in most studies vascular risk factors were only included as covariates. Higher HbA1c levels, higher fasting glucose levels, insulin treatment and longer disease duration might be associated with smaller brain volumes in patients with T2DM.[15–17,23,25,28]

In patients with T2DM, atrophy was related to modest cognitive decrements, particularly for the domains information processing speed and attention and executive functioning.[24] Impaired memory have been associated with subcortical atrophy.[32,33]

Hippocampal atrophy in T2DM

Atrophy of the medial temporal lobe, in particular the hippocampus, is thought to be a marker of neurodegeneration, particularly apparent in Alzheimer's disease.[34] Hippocampal atrophy has been suggested to occur also in patients with T2DM.

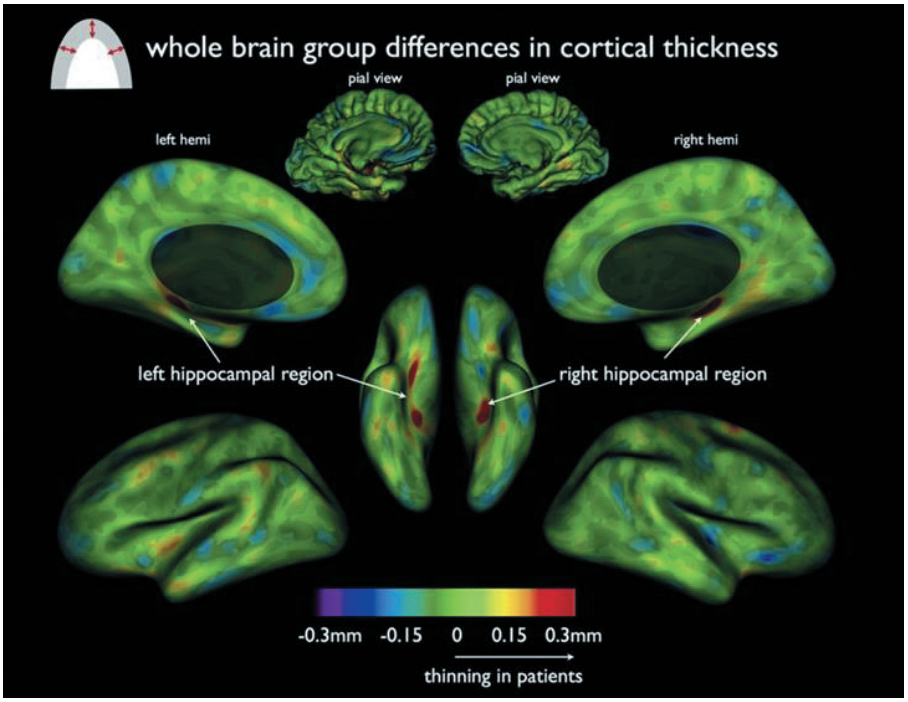


Figure 1 Between-group absolute differences in cortical thickness between patients with T2DM and controls. Region specific changes are visible in the hippocampal regions (Brundel et al. 2010)

Several studies have been published on this topic, including large cohorts, e.g. the LADIS study,[35] the Rotterdam Study,[36] and the Honolulu Asia Aging Study.[16] Hippocampal volume was measured with the medial temporal atrophy (MTA) score,[35] manually traced,[16,28,36-38] or with an automated segmentation method,[14,33,39,40] all at lower/regular field strength (0.5-1.5 tesla). In both middle-aged[28,33,40] and older patients,[16,35,36] the presence of T2DM was associated with smaller hippocampi. Decreased cortical thickness of the medial temporal lobe in patients with T2DM compared to non-diabetic controls has been reported (Figure 1).[39]

Correspondingly, in non-diabetic subjects, significantly smaller hippocampi were found in subjects with impaired glucose tolerance,[37] insulin resistance,[38] or higher fasting glucose levels.[41]

Importantly, most of these studies corrected for head size or intracranial volume,[16,28,33,36,40], but did not correct for total brain volume. Therefore, most studies did not assess if hippocampal atrophy was out of proportion to global brain atrophy. The only study that adjusted hippocampal volumes for total brain volume found no relation between T2DM and hippocampal atrophy.[14]

White matter hyperintensities

White matter hyperintensities (WMHs) are mostly bilateral and symmetrical hyperintense lesions on T2-weigthed MR images.[4] They are distributed in the periventricular and deep white matter of the cerebral hemispheres, in the basal ganglia, and, less frequently, infratentorial. WMHs are a common finding in the general population. The occurrence and degree increase with age, with prevalences of about two-third in subjects aged 60-70, approaching 100% above the age of 80 years.[42] They are strongly correlated with cerebrovascular disease and vascular risk factors, although postmortem studies showed that the underlying histopathology is heterogeneous.[43] They are associated with cognitive, neurological and functional symptoms such as walking difficulties and depression.[44,45]

WMHs can be assessed with visual rating scales, such as the Fazekas scale, Scheltens scale[46] and age-related white matter changes (ARWMC) scale.[47] These scales range from 3 levels to more detailed scales with 36 levels in which WMH are visually quantified in multiple brain regions. It has been reported that WMH rating scale performance varies with WMH prevalence, and therefore with patient groups.[48] In the past ten years, automated segmentation methods have been developed to measure WMH volume more precisely, based on the earlier described classification of voxels on MR images.

White matter hyperintensities in T2DM

The association between T2DM and WMHs is a topic of some controversy. Several studies have investigated the amount of WMHs in patients with T2DM, in community-based cohorts,[14-18,49,50] in patients with clinical vascular disease,[25,31] or in case-control studies.[21,22,24] WMHs have been established with relative crude visual rating scales,[15,16] more comprehensive visual rating scales [18,21,24] or with an automated segmentation method.[14,17,25,49,50]

In middle-aged subjects (with a mean age up to 65 years), a relationship between T2DM and degree of WMHs was reported in a case control study (including assessment with an automated segmentation method)[22,24] and in a clinical based cohort;[31] however, in four population-based cohorts, three of which used automated segmentation methods, no relationship between T2DM and the amount of WMH was found independent of other cardiovascular risk factors.[15,17,49,50]

In older subjects (with a mean age of 65-83 years), more WHMs were reported in patients with T2DM compared with controls,[21] but others did not find this association.[14,16,18,25] In non-diabetic individuals, higher HbA1c levels were associated with a higher degree of visually rated WMHs.[51]

The results of longitudinal studies on the relation between T2DM and WMH are also heterogeneous. Two studies showed no accelerated progression of WMH volumes,[20,25] whereas other population-based studies reported a significantly increased WMH progression rate[26,52,53] in patients with T2DM relative to non-diabetic controls.

Regarding possible determinants of larger WMH volumes in patients with T2DM, higher insulin resistance [54] and HbA1c levels [21] were reported, but not invariably.[22]

Cerebral infarcts

Cerebral infarcts can be subdivided in small subcortical infarcts (counting for about 25% of all ischemic strokes) or large vessel infarcts. Recent small subcortical infarcts are visible on T2-weighted MR images as hyperintense lesions in the territory of one perforating arteriole (generally not exceeding 15 mm in length, but in the acute phase up to 20 mm). Neuroimaging evidence suggest that they can evolve over time in either a white matter hyperintensity or a lacunar cavity (on T2-weighted MRI seen as hypointense lesion) or disappear. [4] Large vessel infarcts are visible on MRI as focal hyperintense lesions on T2-weighted images with corresponding hypointense lesions on T1-weighted imaging, in the territory of a large vessel. Neuropathological correlates suggest that small subcortical infarcts are associated

with occlusion of small arteries or arterioles; large vessel infarcts can be caused by low flow or by embolism from one of the larger cerebral vessels. Brain infarcts are associated with deficits in physical en cognitive function and with depression and dementia. [55,56] Brain infarcts are generally rated visually on MRI.

Cerebral infarcts in T2DM

Many studies have investigated the relationship between T2DM and infarcts on brain MRI, involving population-based cohorts (including the Rotterdam Scan Study and the Cardiovascular Health Study),[16-18,57,58] case-control studies,[19,21,24,33] or patients with a clinical lacunar syndrome.[59]

Because T2DM and as well as prediabetic stages are known risk factors for cardiovascular disease, [60] it does not come as a surprise that multiple studies described an association between T2DM and infarcts on MRI, mostly lacunar infarcts.[16,17,24,33,57-59] A meta-analysis published in 2006 including 20 studies on lacunar infarcts on both CT and MRI, found a significant association between diabetes and lacunar infarcts in general cohorts, vascular cohorts and outpatient cohorts (OR (95% CI) 1.3 (1.1-1.6), 2.2 (1.9-2.5), and 1.4 (1.1-1.8) respectively).[61] Subsequently published studies show similar results.60 In a large study, there was a relationship between the metabolic syndrome and silent brain infarcts.[62] In subjects with the metabolic syndrome, elevated fasting glucose levels were significantly associated with silent brain infarcts.[62,63] In longitudinal studies, this evidence is inconsistent: some studies indicate that T2DM is associated with incident infarcts,[27,31,58] whereas others did not find such an association.[25,64] In non-diabetic subjects, higher insulin resistance and fasting glucose levels >7.1 mmol/L[65] and impaired glucose tolerance[66] were reported to be associated with a higher risk of incident infarcts.

Diabetes duration,[16,17] insulin resistance,[67] nephropathy[68] and higher blood pressure[69,70] are suggested to be determinants of the increased occurrence of brain infarcts on MRI in people with T2DM.

Among patients with T2DM, brain infarcts have been related to modest cognitive decrements, particularly for the domains information processing speed and attention and executive functioning.[24,71]

Microbleeds

Microbleeds are small areas of signal void seen on T2-weighted MRI or other sequences that are sensitive to susceptibility effects.[4] Histopathological studies indicate that these*

lesions reflect haemosiderin-laden macrophages in perivascular tissue consistent with vascular leakage of blood cells.[72] Microbleeds are associated with vascular risk factors and other markers of small vessel disease.[73] Furthermore, they have been related to cognitive impairment and (Alzheimer's) dementia.[74] Different scales are developed to rate brain microbleeds, such as the Microbleed Anatomical Rating Scale (MARS)[75] and the Brain Observer MicroBleed Scale (BOMBS).[76] The chance of detecting microbleeds on MRI is dependent of scan protocol and field strength.[77]

Microbleeds in T2DM

A recent study reported no difference in microbleed occurrence between patients with T2DM and controls (4 and 6 % respectively).[33] A few studies showed diabetes mellitus to be associated with microbleeds in the general population (OR 2.2, 95% CI 1.2-4.2).[74]

EMERGING TECHNIQUES IN T2DM

Diffusion tensor imaging

Diffusion tensor imaging (DTI) assesses structural properties of the cerebral white matter. The technique is based on differences in the diffusion of water molecules. Without barriers, water molecules move in all directions, resulting in isotropic diffusion. In the presence of for example nerve fibers, the diffusion rate is larger in one direction than the other (anisotropic diffusion). The directionality and magnitude of random water movement in tissue can be valued with multiple quantitative measures, e.g. mean diffusivity, transverse or radial diffusivity, axial diffusivity, and the degree of diffusion anisotropy (fractional anisotropy). Changes in axial diffusivity might be associated with axonal injury, whereas transverse diffusivity alterations may be related to demyelination.[78] These values can be measured both globally and regionally. Moreover, with tractography, based on measurement of the directionality of the diffusion, white matter tracts can be determined to visualize the brain as a network (Figure 2).

DTI is a sensitive technique to study subtle white matter changes.[79] In healthy older individuals, brain network properties measured with DTI have been shown to be related to WMHs and cognitive functioning.[80,81]

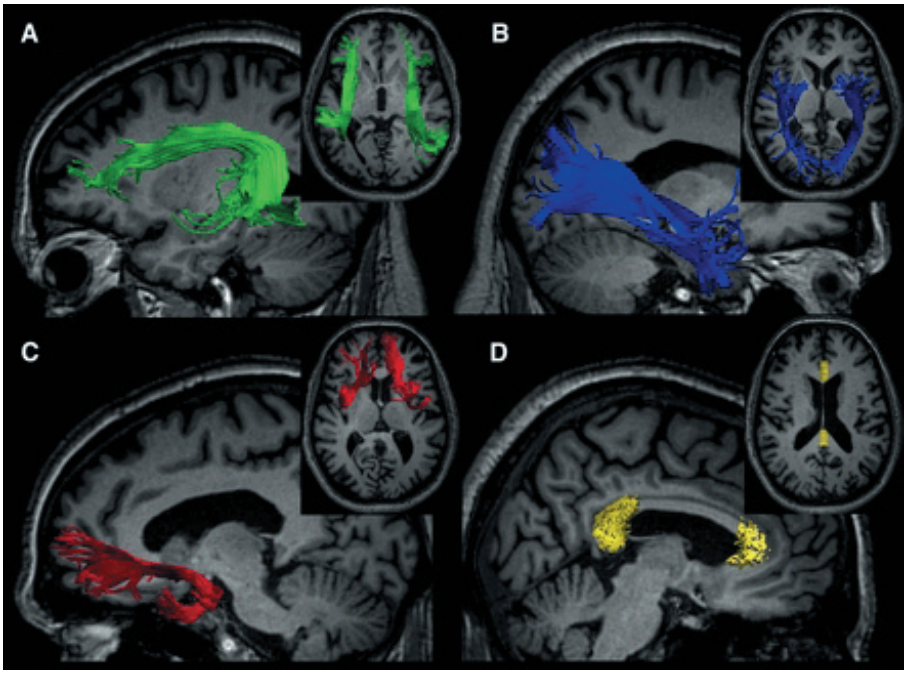


Figure 2 Reconstructed white matter tracts using DTI (Reijmer et al., 2013)

DTI in T2DM

A number of case-control studies, investigating adolescents, middle-aged as well as older subjects, mostly without significant diabetes-related complications, have been published over the last few years. They consistently showed differences in DTI measures between patients and controls: fractional anisotropy is decreased in one or more regions, in adolescents, middle-aged and older patients with T2DM compared with controls.[14,82-85] The frontal lobes seem to be specific vulnerable areas.[82-84] Mean diffusivity values were reported to be increased in T2DM patients in three studies.[14,82,83] This was based on predominantly increased transverse diffusivity (suggesting myelination changes)[82] or changes in diffusivity in both directions.[83] With tractography, alterations in local and global network properties were found in patients with T2DM.[86]

Correspondingly, adolescents with the metabolic syndrome showed reductions of microstructural integrity of the white matter.[29] Within patients with T2DM, differences in DTI measures were found to be associated with HbA1C levels[83] and diabetes duration.[82] DTI abnormalities in T2DM strongly correlate with cognitive performance.[83,86]

Functional neuroimaging: fMRI and PET

With functional MRI (fMRI) and positron emission tomography (PET), it is possible to show changes in regional brain activity. These techniques are based on increased neuronal metabolism in activated neurons. This results in regional increases in oxygen demand, and consequently increases in perfusion (fMRI) and glucose uptake or metabolism (PET).

In fMRI the blood-oxygen-level-dependent (BOLD) contrast is most commonly used, which is based on differences in MR signal between oxygenated and deoxygenated blood. Studies can be performed on 1. Background patterns (resting-state fMRI: an important background pattern is the default mode network (DMN), which includes the posterior cingulate cortex and temporoparietal posterior association cortical regions. The DMN is activated in rest and suppressed during cognitive activity; 2. Signal changes in certain areas that are caused by active tasks during scanning. It is possible to determine which brain regions are functionally connected (Figure 3). In T2DM, neurovascular coupling (i.e. the processes by which neural activity influences the haemodynamic properties of the surrounding vasculature) might be altered.[87] This may influence the BOLD signal and makes the interpretation of fMRI data more difficult.

PET is a quantitative radionuclide imaging technique based on the detection of a peripherally injected radiolabelled positron-emitting tracer as it passes through or accumulates in the brain. In diabetes research, mostly ^{18}F-fluorodeoxyglucose (FDG) is used, a glucose analogue. Its uptake is affected by blood glucose levels, and therefore the sensitivity of FDG-PET might be lower in hyperglycemia.

fMRI in T2DM

fMRI studies in T2DM are scarce. Three small case-control studies performed resting state fMRI in patients with T2DM (disease duration 1-10 years) compared with controls. They show reduced functional connectivity between bilaterally widespread regions, including the hippocampus and other regions of the DMN.[88-90] Within T2DM patients, insulin resistance (defined by HOMA-IR)[88] and HbA1c levels[89] were inversely correlated with functional connectivity in several brain regions in resting state. One study reported reduced activation intensity in multiple brain regions during a verbal working memory task in individuals with the metabolic syndrome.[91]

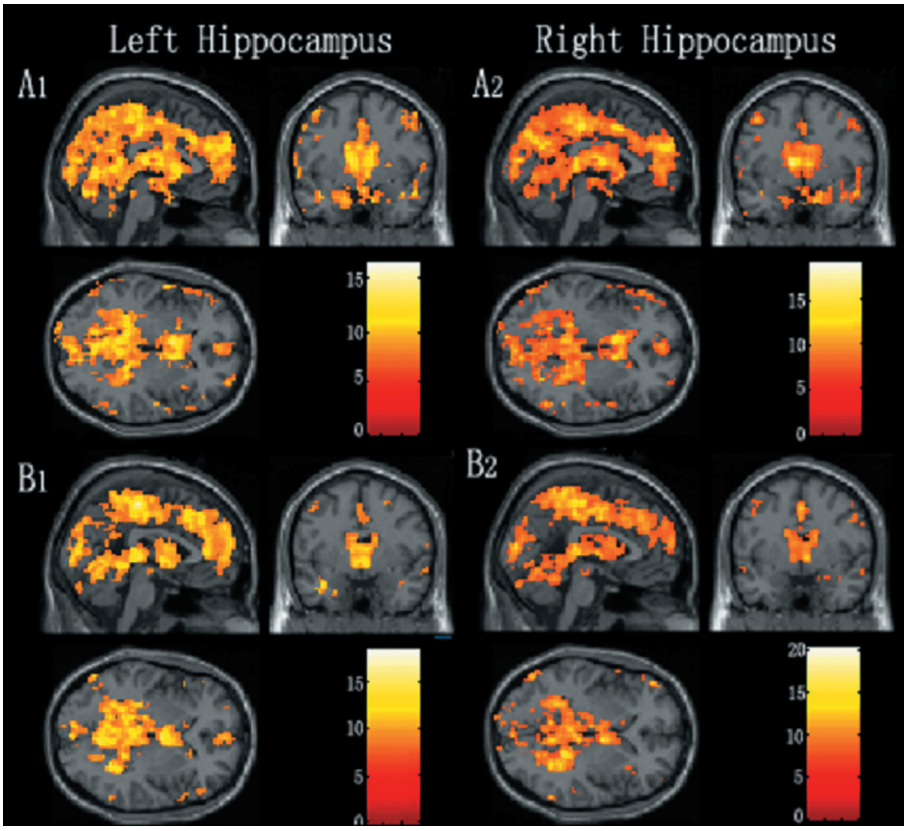


Figure 3 fMRI study showing hippocampal functional connectivity for healthy controls (A1 and A2) and T2DM patients (B1 and B2) (Zhou et al., 2010)

PET in T2DM

Few functional imaging studies with FDG-PET in patients with T2DM have been published. They report on small case-control studies (a maximum of 23 patients with (pre-)diabetes was investigated). One study reported decreased glucose metabolism in multiple brain areas in patients with T2DM and cerebral microangiopathy (a clinical lacunar infarct and WMH on brain imaging) compared with non-diabetic patients with cerebral microangiopathy and healthy controls, but significant reductions in the diabetic group were due only to brain atrophy, and not to diabetes.[92] Another study found increased insulin resistance (defined by a higher HOMA-IR) to be associated with reduced glucose metabolism in an Alzheimer-like pattern of brain regions in patients with pre-diabetes or newly diagnosed T2DM, compared with non-diabetic subjects. Furthermore, patients with insulin resistance

performed worse on a memory task and showed a more widespread brain activation, suggesting a compensatory mechanism due to dysfunction of the neuronal network normally active in memory.[93] In subjects above the age of 55 with normal cognitive performance to mild dementia, diabetes was found to be independently associated with lower glucose metabolism in multiple brain regions.[94]

Cerebral blood flow and cerebrovascular reactivity

Cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) are measurements for cerebral microvascular function, that can be assessed with different techniques, both invasively and non-invasively:

1. ***Single-photon emission computed tomography** (SPECT) is a nuclear imaging technique based on the detection of a peripherally injected radiolabelled positron-emitting tracer radioisotope. Frequently used tracers as markers of blood flow are N-isopropyl-^{123}I-iodoamphetamine (^{123}I-IMP) and 99mTc-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO). With SPECT it is possible to assess blood flow regionally (Figure 4).*
2. *With **MRI**, two techniques are available to measure perfusion non-invasively. Phase-contrast magnetic resonance angiography (PC-MRA) is a fast method to measure quantitative volume flow in the major cerebral arteries, based on differences in dephasing of moving spins in proportion to their velocity. It gives only information about global cerebral blood flow. With arterial spin labeling (ASL), the hydrogen nuclei of the arterial blood are labeled by magnetic inversion. An image of a region of interest is acquired, which includes the labeled blood. Secondly, a control image is acquired, and with image subtraction the perfusion of the region of interest can be measured.[95]*
3. ***Transcranial Doppler** (TCD) is a quick and inexpensive test which measures the velocity of blood flow through larger brain vessels with ultrasound.*

Cerebrovascular reactivity (CVR) is a measure for the vasodilatory ability of the cerebral microvasculature. It is defined as the mean increase in blood flow (SPECT, ASL) or blood flow velocity (TCD) after stimulation with either acetazolamide or CO_2, which both induce dilation of the cerebral microarteriolar vessels, with consequently an increased blood flow through these vessels.

Whether lower cerebral blood flow or cerebrovascular reactivity is associated with cognitive performance in healthy individuals, remains unclear, particularly in the presence of brain atrophy, since it is not distinct if this association is mediated or confounded by brain atrophy.[96,97]

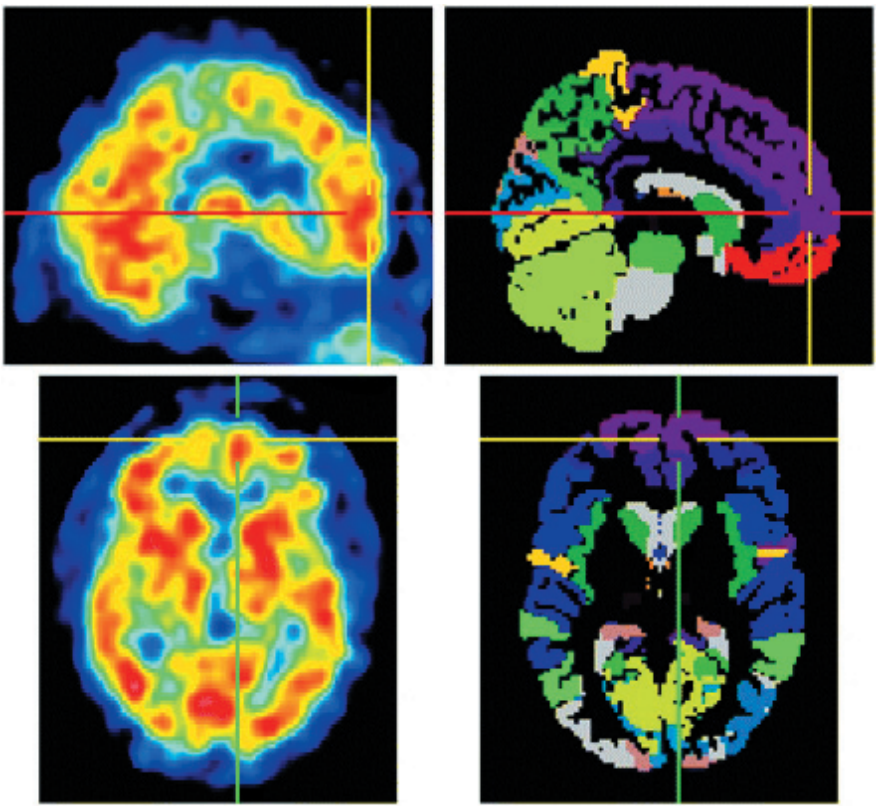


Figure 4 Regional cerebral blood flow on sagittal and transversal SPECT slices of a patient with T2DM (left) with corresponding slices of International Consortium for Brain Mapping template (right) (Kaplar et al., 2009)

Cerebral blood flow in T2DM

Studies on global cerebral blood flow in T2DM, all with a case-control design, show conflicting results. Two studies with PC-MRA and one study with TCD (mean age of assessed patients 57-69 years) did not find any differences between T2DM patients and controls.[98-100] These studies concerned both newly diagnosed and relatively well-controlled T2DM patients[99,100] as well as patients with longer disease duration.[98] A decreased mean blood flow velocity (measured with TCD) has been reported in T2DM patients with advanced complications.[101]

With SPECT, cerebral perfusion has been assessed both globally and regionally. Three small studies have been performed, pointing to the same direction: both global and regional blood flow is diminished in patients with T2DM compared with controls.[92,102,103] Regional differences were found in the frontal and parietal lobes.[103] Another study on regional differences in CBF measured with ASL-MRI, showed reduced CBF in all

brain regions in patients with T2DM (without significant complications) compared with controls.[23] It should be noted that not all studies corrected their CBF measures for brain atrophy.[99,101-103] The reported associations between diabetes and CBF might therefore only be an 'artefact' due to cerebral atrophy. In fact, significant reductions in CBF did not remain significant when corrected for atrophy in one study.[92]

In patients with T2DM, higher HbA1c[23,101] and higher systolic blood pressure[23] were reported to be associated with lower CBF.

Total CBF was associated with cognitive performance in one study, but this association was not affected by T2DM.100 Total CBF was also not associated with cognitive performance in T2DM patients after four years in the same study population.[104]

Cerebrovascular reactivity in T2DM

Studies on relatively well-controlled patients with T2DM showed no difference in CVR (measured with TCD[105] and PC-MRA[99]) between T2DM patients and controls. On the other hand, in selected subgroups of patients with long disease duration or advanced complications, a decreased CVR, measured with TCD[98,101,106] and ASL-MRI,[23] was reported.

Within patients with T2DM, higher HbA1C levels,101 retinopathy,[23,101] longer (>10 years) disease duration,[98] and hypertension[23] were reported to be associated with lower CVR (although others did not find an association with higher HbA1c,[107] disease duration,[107]or dyslipidemia[23]).

Magnetic resonance spectroscopy

Proton MR spectroscopy (^{1}H-MRS) is a noninvasive technique to determine the resonance peaks of brain metabolites and neurotransmitters, mostly N-acetyl-L-aspartate (NAA), choline (Cho), creatine (Cr), and myo-inositol (mI). NAA is located only in neurons and their axons, and reduced NAA is therefore an indicator of neuronal dysfunction, for example in neurodegenerative diseases. Cho is predominantly distributed in glial cells, and is involved in cell membrane metabolism and myelin formation (increased levels are associated with gliosis or necrosis). Cr plays a role in cellular energy metabolism, and tends to remain relatively unchanged. Therefore, Cr is often used as an internal standard against which the other metabolites can be related. Finally, mI is a metabolite derived from glucose, that can be found in astrocytes and is considered a glial cell marker.[108] Changes in resonance peaks of mentioned metabolites are regularly determined in one or a few brain regions, depicted by manually delineated voxels.

MRS in T2DM

A limited number of studies, all with a case-control design, examined cerebral metabolism with 1H-MRS, mostly in small patient groups (a maximum of 72 diabetic patients was investigated). A large proportion of studies investigated relatively young patients (<60 years).[109-112] Diabetes duration varied widely between and within studies, from one week to 44 years. All used 1.5 Tesla field strength MRI. They explored different brain areas, both cortical and subcortical, which make the studies difficult to compare.

All studies investigated Cho levels, and four of them found increased Cho levels or an increased Cho/Cr ratio: in the occipital gray matter,[110,111,113] the parietal white matter[113] and the lenticular nuclei.[114] Higher Cho levels were found even in individuals with impaired glucose tolerance.[112] Higher HbA1C levels[112] and peripheral polyneuropathy110 were found to be associated with higher Cho/Cr ratios. However, three studies did not find a relationship between diabetes and Cho levels,[109,115] or even found an inverse relationship.[112] The metabolite NAA was also frequently investigated, and most studies showed decreased levels of NAA/Cr ratios, in the parietal white matter,[113] in the lenticular nuclei (only on the left side),[114] and in the left deep white matter area,[99] suggesting neuronal dysfunction. On the other hand, others showed no association between diabetes and NAA levels[109,111,115] or only higher NAA levels in the frontal cortex in T2DM patients with poor glycemic control compared to T2DM patients with better glycemic control.[112] The studies that investigated the metabolite mI have consistently found a significant increase in mI levels or mI/Cr ratio, in the frontal white matter [109] and cortex,[112] the occipital cortex,[110,113] parietal white matter110 and cortex.[113] One study investigated the relationship between brain metabolites and cognitive functioning in patients with T2DM. They observed no significant associations between metabolite ratios of NAA/Cr, Cho/Cr and NAA/Cho and cognitive functioning.[115]

SYNTHESIS

T2DM is associated with structural and functional changes in the brain, which are usually only mildly distinct from normal aging (Table 1). A modest degree of global brain atrophy - an equivalent of 3-5 years of normal brain aging - and a slightly increased atrophy rate have been consistently reported, in all age groups, already visible in prediabetic stages. Hippocampal atrophy is proportional to global brain atrophy in patients with T2DM. Lacunar infarcts are 1.5-2 times more often

found than in non-diabetic subjects. Inconsistent results with respect to WMH, another marker of small vessel disease, have been published, both in cross-sectional and longitudinal studies. Differences in the measurement of WMHs may in part explain this inconsistency. Another explanation might be the large inter-individual differences in WMH volumes. Therefore, group differences in WMH volumes are more difficult to demonstrate, and cohorts need to be larger. DTI assesses white matter structural properties in a different way, and is probably sensitive to more subtle white matter changes. Microstructural abnormalities may be an early marker for cerebral brain damage in patients with T2DM. DTI is a novel technique, and the results should be interpreted with caution, since the functional connectivity of the brain is not completely understood.

Studies on cerebral blood flow and cerebrovascular reactivity in patients with T2DM show conflicting results. Some studies found a relationship between T2DM and diminished brain perfusion, mostly in studies with longer disease duration or more advanced complications. In most of the studies no correction for brain atrophy was made. This may have confounded the assessment of brain perfusion. Since only cross-sectional studies are available, it is uncertain if changes in cerebral blood flow are the cause or consequence of alterations in cerebral function in T2DM. Reverse causality might play a role: the demand for CBF may be relatively lower in abnormal atrophic brain tissue.

Only limited data on functional imaging and spectroscopy are available in patients with T2DM. No definite conclusion can be drawn on these results. Nevertheless, functional and metabolic changes might serve as an early marker of diabetes-related brain damage, but obviously need to be explored in larger patient groups.

Higher field strength increases resolution of MRI and consequently the accuracy of measurements. Ultra-high field (7 Tesla) MRI, which is currently not used in daily practice, will provide detailed information on brain damage. It additionally gives the opportunity to visualize microvascular brain lesions (i.e. microbleeds and microinfarcts) and explore their relationship with T2DM. Microinfarcts have been found as an important biomarker of dementia in postmortem studies.[116,117] With advanced imaging techniques at ultra-high field strength, these lesions have recently been visualized *in vivo* (figure 5).[118]

The molecular mechanisms underlying brain damage related to T2DM remains unclear. T2DM has been associated with different pathophysiological processes involved in both vascular (e.g. endothelial dysfunction, pro-inflammatory and prohrombotic factors3) and neurodegenerative pathways (e.g. alterations in the phosphorylation of tau[119] and decreased breakdown of amyloid[120,121]). The brain

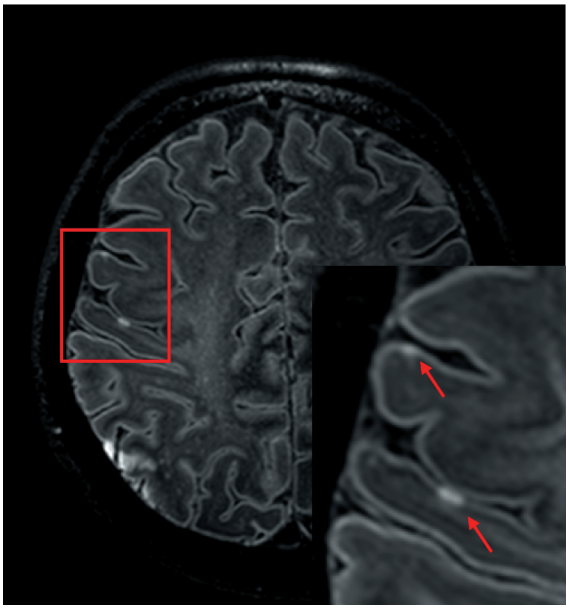


Figure 5 Example of minute acute cortical ischemic lesions on 7 Tesla FLAIR images (Brundel et al, 2012)

imaging studies, reviewed in this paper, show that T2DM is associated with both degenerative and vascular brain damage. With respect to a vascular etiology it is important to also identify the role of other vascular risk factors than T2DM. Only a small number of previous studies have looked at possible interactions between T2DM and other vascular risk factors in relation to brain imaging abnormalities. Thus far relatively few studies investigated the relationship between brain imaging abnormalities and cognitive performance in T2DM. An association between atrophy, brain infarcts and DTI measures and cognitive impairment has been suggested, but further studies are needed to explore this.

FUTURE PERSPECTIVES

T2DM is probably associated with both neurodegenerative and vascular disease, although these associations are rather heterogeneous and non-specific. Most patients will show only mild brain changes compared with normal aging. However, some patients with T2DM, particularly over the age of 60-70 years, may suffer from more severe progressive brain abnormalities and cognitive decline. Longitudinal

studies may yield imaging markers that help to identify these patients. Longitudinal data, and precise risk factor assessment are necessary to get insight in the course of brain damage in T2DM and underlying mechanisms. Since vascular risk factor profile and dementia risk change over time, timing of assessment (i.e. in early disease stages before midlife) is important, also in the light of potential early interventions. Combining structural and functional MRI will offer probably the most sensitive biomarkers for brain damage in T2DM. Finally, more advanced MR imaging techniques and higher field strength will increase detection of subtle brain damage in T2DM patients. It may then also be possible to explore the suitability of these biomarkers as therapeutic trial outcome measures.

Table 1 Summary of available evidence on different imaging techniques in T2DM and prediabetic stages

	Number of cohorts	Populations
Brain atrophy and vascular lesions		
Brain atrophy	>25	Community-based, clinical-based, case-control
WMH	>20	Community-based, clinical-based, case-control
Infarcts	>25	Community-based, clinical-based, case-control
Microbleeds	5	Population-based, case-control
Emerging techniques		
DTI	7	Case-control
Functional MRI	7	Case-control
CBF	8	Case-control
CVR	8	Case-control
Spectroscopy	8	Case-control

Findings in patients with T2DM compared with controls	Possible determinants	Evidence
Modest brain atrophy in all age groups, already in prediabetic stages (equivalent of 2-5 years of normal aging). Hippocampal atrophy not disproportionate	Hypertension, HbA1c, insulin levels, disease duration	Strong
Only lsightly increased WMH volumes	HbA1c, insulin resistance	Strong
Occurrence of infarcts 1.3-2.2 times increased, especially lacunar infarcts, in middle-aged and older patients	Disease duration	Strong
Prevalence of microbleeds comparable or increased	-	Weak
↓ fractional anisotropy, ↑ mean diffusivity, alterations in local and global network properties	HbA1c, disease duration	Moderate
Inconsistent results; mostly reduced widespread functional connectivity and decreased metabolism	Insulin resistance	Weak
Inconsistent results	HbA1c, blood pressure	Weak
Diminished CVR in patients with advanced disease	HbA1c, disease duration, complications	Moderate
↑ myo-inositol; inconsistent results for NAA and Cho	HbA1c, complications	Moderate

REFERENCES

1. International Diabetes Federation, 2013. IDF Diabetes Atlas, sixth ed. International Diabetes Federation, Brussels, Belgium.
2. Luitse MJ, Biessels GJ, Rutten GE, Kappelle LJ.Diabetes, hyperglycaemia, and acute ischaemic stroke. *Lancet Neurol* 2012;11:261-271.
3. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. *Lancet Neurol* 2006;5:64-74.
4. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, Barkhof F, Benavente OR, Black SE, Brayne C, Breteler M, Chabriat H, Decarli C, de Leeuw FE, Doubal F, Duering M, Fox NC, Greenberg S, Hachinski V, Kilimann I, Mok V, Van Oostenbrugge R, Pantoni L, Speck O, Stephan BC, Teipel S, Viswanathan A, Werring D, Chen C, Smith C, van Buchem M, Norrving B, Gorelick PB, Dichgans M. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* 2013;12:822-838.
5. Jagust WJ, Zheng L, Harvey DJ, Mack WJ, Vinters HV, Weiner MW, Ellis WG, Zarow C, Mungas D, Reed BR, Kramer JH, Schuiff N, DeCarli C, Chui HC. Neuropathological basis of magnetic resonance images in aging and dementia. *Ann Neurol* 2008;63:72-80.
6. Resnick SM, Pham DL, Kraut MA, Zonderman AB, Davatzikos C. Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. *J Neurosci* 2003;15:3295-3301.
7. Scahill RI, Frost C, Jenkins R, Whitwell JL, Rossor MN, Fox NC. A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Arch Neurol* 2003;60:989-994.
8. Appelman AP, Exalto LG, van der GY, Biessels GJ, Mali WP, Geerlings MI. White matter lesions and brain atrophy: more than shared risk factors? A systematic review. *Cerebrovasc Dis* 2009;28:227-242.
9. Kloppenborg RP, Nederkoorn PJ, Grool AM, Vincken KL, Mali WP, Vermeulen M, van der Graaf Y, Geerlings MI. Cerebral small-vessel disease and progression of brain atrophy: the SMART-MR study. *Neurology* 2012;13:2029-2036.
10. Salat DH, Kaye JA, Janowsky JS. Greater orbital prefrontal volume selectively predicts worse working memory performance in older adults. *Cereb Cortex* 2002;12:494-450.
11. Gomori JM, Steiner I, Melamed E, Cooper G. The assessment of changes in brain volume using combined linear measurements. A CT-scan study. *Neuroradiology* 1984;26:21-24.
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 ageing: diagnostic value and neuropsychological correlates. *J Neurol Neurosurg Psychiatry* 1992;55:967-972.

13. Jongen C, Biessels GJ. Structural brain imaging in diabetes: a methodological perspective. *Eur J Pharmacol* 2008;585:208-218.
14. Falvey CM, Rosano C, Simonsick EM, Harris T, Strotmeyer ES, Satterfield S, Yaffe K. Macro- and microstructural magnetic resonance imaging indices associated with diabetes among community-dwelling older adults. *Diabetes Care* 2013;36:677-682.
15. Knopman DS, Mosley TH, Catellier DJ, Sharrett AR. Cardiovascular risk factors and cerebral atrophy in a middle-aged cohort. *Neurology* 2005;27:876-881.
16. Korf ES, White LR, Scheltens P, Launer LJ. Brain aging in very old men with type 2 diabetes: the Honolulu-Asia Aging Study. *Diabetes Care* 2006;29:2268-2274.
17. Saczynski JS, Siggurdsson S, Jonsson PV, Eiriksdottir G, Olafsdottir E, Kjartansson O, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Glycemic status and brain injury in older individuals: the age gene/environment susceptibility-Reykjavik study. *Diabetes Care* 2009;32:1608-1613.
18. Schmidt R, Launer LJ, Nilsson LG, Pajak A, Sans S, Berger K, Breteler MM,de Ridder M, Dufouil C, Fuhrer R, Giampaoli S, Hofman A. Magnetic resonance imaging of the brain in diabetes: the Cardiovascular Determinants of Dementia (CASCADE) Study. *Diabetes* 2004;53:687-692.
19. Araki Y, Nomura M, Tanaka H, Yamamoto H, Yamamoto T, Tsukaguchi I. MRI of the brain in diabetes mellitus. *Neuroradiology* 1994;36:101-103.
20. De Bresser J, Tiehuis AM, van den Berg E, Reijmer YD, Jongen C, Kappelle LJ, Mali WP, Viergever MA, Biessels GJ. Progression of cerebral atrophy and white matter hyperintensities in patients with type 2 diabetes. *Diabetes Care* 2010;33:1309-1314.
21. Van Harten B, Oosterman JM, Potter van Loon BJ, Scheltens P, Weinstein HC. Brain lesions on MRI in elderly patients with type 2 diabetes mellitus. *Eur Neurol* 2007;57:70-74.
22. Jongen C, van der Grond J, Kappelle LJ, Biessels GJ, Viergever MA, Pluim JP. Automated measurement of brain and white matter lesion volume in type 2 diabetes mellitus. *Diabetologia* 2007;50:1509-1516.
23. Last D, Alsop DC, Abduljalil AM, Marquis RP, de Bazelaire C, Hu K, Cvallerano J, Novak V. Global and regional effects of type 2 diabetes on brain tissue volumes and cerebral vasoreactivity. *Diabetes Care* 2007;30:1193-1199.
24. Manschot SM, Brands AM, van der Grond J, Kessels RP, Algra A, Kappelle LJ, Biessels GJ. Brain magnetic resonance imaging correlates of impaired cognition in patients with type 2 diabetes. *Diabetes* 2006;55:1106-1113.
25. Van Elderen SG, de Roos A, de Craen AJ, Westendorp RG, Blauw GJ, Jukema JW, Bollen EL, Middelkoop HA, van Buchem MA, van der Grond J. Progression of

brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. *Neurology* 2010;14:997-1002.

26. Espeland MA, Bryan RN, Goveas JS, Robinson JG, Siddiqui MS, Liu S, Hogan PE, Casanova R, Coker LH, Yaffe K, Masaki K, Rossom R, Resnick SM. Influence of type 2 diabetes on brain volumes and changes in brain volumes: results from the Women's Health Initiative Magnetic Resonance Imaging studies. *Diabetes Care* 2013;36:90-97.
27. Knopman DS, Penman AD, Catellier DJ, Coker LH, Shibata DK, Sarrett AR, Mosley TH Jr. Vascular risk factors and longitudinal changes on brain MRI: the ARIC study. *Neurology* 2011;31:1879-1885.
28. Bruehl H, Sweat V, Tirsi A, Shah B, Convit A. Obese Adolescents with Type 2 Diabetes Mellitus Have Hippocampal and Frontal Lobe Volume Reductions. *Neurosci Med* 2011;1:34-42.
29. Yau PL, Castro MG, Tagani A, Tsui WH, Convit A. Obesity and metabolic syndrome and functional and structural brain impairments in adolescence. *Pediatrics* 2012;130:e856-e864.
30. Enzinger C, Fazekas F, Matthews PM, Ropele S, Schmidt H, Smith S, Schmidt R. Risk factors for progression of brain atrophy in aging: six-year follow-up of normal subjects. *Neurology* 2005;24:1704-1711.
31. Kooistra M, Geerlings MI, Mali WP, Vincken KL, van der Graaf Y, Biessels GJ. Diabetes mellitus and progression of vascular brain lesions and brain atrophy in patients with symptomatic atherosclerotic disease. The SMART-MR study. *J Neurol Sci* 2013;15:69-74.
32. Akisaki T, Sakurai T, Takata T, Umegaki H, Araki A, Mizuno S, Tanaka S, Ohashi Y, Iguchi A, Ykono K, Hideki I. Cognitive dysfunction associates with white matter hyperintensities and subcortical atrophy on magnetic resonance imaging of the elderly diabetes mellitus Japanese elderly diabetes intervention trial (J-EDIT). *Diabetes Metab Res Rev* 2006;22:376-384.
33. Moran C, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Munch G, Wood AG, Forbes J, Gerenaway TM, Pearson S, Srikanth V. Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. *Diabetes Care* 2013;36:4036-4042.
34. Scheltens P, Fox N, Barkhof F, De Carli C. Structural magnetic resonance imaging in the practical assessment of dementia: beyond exclusion. *Lancet Neurol* 2002;1:13-21.
35. Korf ES, van Straaten EC, de Leeuw FE, van der Flier WM, Barkhof F, Pantoni L, Basile AM, Inzitari D, Erkinjuntti T, Wahlund LO, Rostrup E, Schmidt R, Fazekas F, Scheltens P. Diabetes mellitus, hypertension and medial temporal lobe atrophy: the LADIS study. *Diabet Med* 2007;24:166-171.
36. Den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MMB. Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia* 2003;46:1604-1610.

37. Convit A, Wolf OT, Tarshish C, de Leon MJ. Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly. *Proc Natl Acad Sci U S A* 2003;18:2019-2022.
38. Ursache A, Wedin W, Tirsi A, Convit A. Preliminary evidence for obesity and elevations in fasting insulin mediating associations between cortisol awakening response and hippocampal volumes and frontal atrophy. *Psychoneuroendocrinology* 2012;37:1270-1276.
39. Brundel M, van den Heuvel M, de Bresser J, Kappelle LJ, Biessels GJ. Cerebral cortical thickness in patients with type 2 diabetes. *J Neurol Sci* 2010;15:126-130.
40. Gold SM, Dziobek I, Sweat V, Tirsi A, Rogers K, Bruehl H, Tsui W, Richardson S, Javier E, Convit A. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. *Diabetologia* 2007;50:711-719.
41. Cherbuin N, Sachdev P, Anstey KJ. Higher normal fasting plasma glucose is associated with hippocampal atrophy: The PATH Study. *Neurology* 2012;4:1019-1026.
42. De Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J Neurol Neurosurg Psychiatry* 2001;70:9-14.
43. Gouw AA, Seewann A, van der Flier WM, Barkhof F, Rozemuller AM, Scheltens P, Geurts JJ. Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. *J Neurol Neurosurg Psychiatry* 2011;82:126-135.
44. Haley AP, Hoth KF, Gunstad J, Paul RH, Jefferson AL, Tate DF, Ono M, Jerskey BA, Poppas A. Sweet LH, Cohen RA. Subjective cognitive complaints relate to white matter hyperintensities and future cognitive decline in patients with cardiovascular disease. *Am J Geriatr Psychiatry* 2009;17:976-985.
45. Inzitari D, Pracucci G, Poggesi A, Carlucci G, Barkhof F, Chabriat H, Erkinjuntti T, Fazekas F, Ferro JM, Hennerici M, Langhorne P, O'Brien J, Scheltens P, Visser MC, Wahlund LO, Waldemar G, Wallin A, Pantoni L. Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (leukoaraiosis and disability) study cohort. *BMJ* 2009;339:b2477.
46. Scheltens P, Barkhof F, Leys D, Pruvo JP, Nauta JJ, Vermersch P, Steinling M, Valk J. A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. *J Neurol Sci* 1993;114:7-12.
47. 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.
48. Wardlaw JM, Ferguson KJ, Graham C. White matter hyperintensities and rating scales-observer reliability varies with lesion load. *J Neurol* 2004;251:584-590.

49. Jeerakathil T, Wolf PA, Beiser A, Massaro J, Seshadri S, D'Agostino RB, DeCarli C. Stroke risk profile predicts white matter hyperintensity volume: the Framingham Study. *Stroke* 2004;35:1857-1861.
50. Kumar R, Anstey KJ, Cherbuin N, Wen W, Sachdev PS. Association of type 2 diabetes with depression, brain atrophy, and reduced fine motor speed in a 60- to 64-year-old community sample. *Am J Geriatr Psychiatry* 2008;16:989-998.
51. Murray AD, Staff RT, Shenkin SD, Deary IJ, Starr JM, Whalley LJ. Brain white matter hyperintensities: relative importance of vascular risk factors in nondemented elderly people. *Radiology* 2005;237:251-257.
52. Gouw AA, van der Flier WM, Fazekas F, van Straaten EC, Pantoni L, Poggesi A, Inzitari D, Erkinjuntti T, Wahlund LO, Waldemar G, Schmidt R, Scheltens P, Barkhof F. Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. *Stroke* 2008;39:1414-1420.
53. Taylor WD, Steffens DC, MacFall JR, McQuoid DR, Payne ME, Provenzale JM, Krishnan KR. White matter hyperintensity progression and late-life depression outcomes. *Arch Gen Psychiatry* 2003;60:1090-1096.
54. Anan F, Masaki T, Iwao T, Eto T, Shimomura T, Umeno Y, Eshima N, Saikawa T, Yoshimatsu H. The role of microalbuminuria and insulin resistance as significant risk factors for white matter lesions in Japanese type 2 diabetic patients. *Curr Med Res Opin* 2008;24:1561-1567.
55. Marchant NL, Reed BR, Sanossian N, Madison CM, Kriger S, Dhada R, Mack WJ, DeCarli C, Weiner MW, Mungas DM, Chui HC, Jagust WJ. The aging brain and cognition: contribution of vascular injury and abeta to mild cognitive dysfunction. *JAMA Neurol* 2013;70:488-495.
56. Vermeer SE, Longstreth WT Jr, Koudstaal PJ. Silent brain infarcts: a systematic review. *Lancet Neurol* 2007;6:611-619.
57. Longstreth WT Jr, Bernick C, Manolio TA, Bryan N, Jungreis CA, Price TR. Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: the Cardiovascular Health Study. *Arch Neurol* 1998;55:1217-1225.
58. Vermeer SE, Hollander M, van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the Rotterdam Scan Study. *Stroke* 2003;34:1126-1129.
59. Arauz A, Murillo L, Cantu C, Barinagarrementeria F, Higuera J. Prospective study of single and multiple lacunar infarcts using magnetic resonance imaging: risk factors, recurrence, and outcome in 175 consecutive cases. *Stroke* 2003;34:2453-2458.
60. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stempfer M, Stehouwer CD, Lewington S, Pennells L,

Thompson A, Sattar N, White IR, Ray KK, Danesh J. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010;26:2215-2222.

61. Van Harten B, de Leeuw FE, Weinstein HC, Scheltens P, Biessels GJ. Brain Imaging in Patients With Diabetes: A systematic review. *Diabetes Care* 2006;29:2539-2548
62. Kwon HM, Kim BJ, Lee SH, Choi SH, Oh BH, Yoon BW. Metabolic syndrome as an independent risk factor of silent brain infarction in healthy people. *Stroke* 2006;37:466-470.
63. Bokura H, Yamaguchi S, Iijima K, Nagai A, Oguro H. Metabolic syndrome is associated with silent ischemic brain lesions. *Stroke* 2008;39:1607-1609.
64. Van Dijk EJ, Prins ND, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MM. Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan study. *Stroke* 2008;39:2712-2719.
65. Thacker EL, Psaty BM, McKnight B, Heckbert SR, Longstreth WT Jr, Mukamal KJ, Meigs JB, de Boer IH, Boyko EJ, Carnethon MR, Kizer JR, Tracy RP, Smith NL, Siscovick DS. Fasting and post-glucose load measures of insulin resistance and risk of ischemic stroke in older adults. *Stroke* 2011;42:3347-3351.
66. Tanizaki Y, Kiyohara Y, Kato I, Iwamoto H, Nakayama K, Shinohara N, Arima H, Tanaka K, Ibayashi S, Fujishima M. Incidence and risk factors for subtypes of cerebral infarction in a general population: the Hisayama study. *Stroke* 2000;31:2616-2622.
67. Matsumoto K, Miyake S, Yano M, Ueki Y, Miyazaki A, Hirao K, Tominaga Y. Insulin resistance and classic risk factors in type 2 diabetic patients with different subtypes of ischemic stroke. *Diabetes Care* 1999;22:1191-1195.
68. Nakamura T, Kawagoe Y, Matsuda T, Ueda Y, Ebihara I, Koide H. Silent cerebral infarction in patients with type 2 diabetic nephropathy. Effects of antiplatelet drug dilazep dihydrochloride. *Diabetes Metab Res Rev* 2005;21:39-43.
69. Eguchi K, Ishikawa J, Hoshide S, Ishikawa S, Shimada K, Kario K. Impact of blood pressure vs. glycemic factors on target organ damage in patients with type 2 diabetes mellitus. *J Clin Hypertens* 2006;8:404-410.
70. Nomura K, Hamamoto Y, Takahara S, Kikuchi O, Honjo S, Ikeda H, Wada Y, Nabe K, Okumra R, Koshiyama H. Relationship between carotid intima-media thickness and silent cerebral infarction in Japanese subjects with type 2 diabetes. *Diabetes Care* 2010;33:168-170.
71. Imamine R, Kawamura T, Umemura T, Umegaki H, Kawano N, Hotta M, Kouchi Y, Hatsuda S, Watarai A, Kanai A, Nakashima E, Sano T, Sakakibara T, Nakamura J, Hotta N. Does cerebral small vessel disease predict future decline of cognitive function in elderly people with type 2 diabetes? *Diabetes Res Clin Pract* 2011;94:91-99.
72. Shoamanesh A, Kwok CS, Benavente O. Cerebral microbleeds: histopathological correlation of neuroimaging. *Cerebrovasc Dis* 2011;32:528-534.

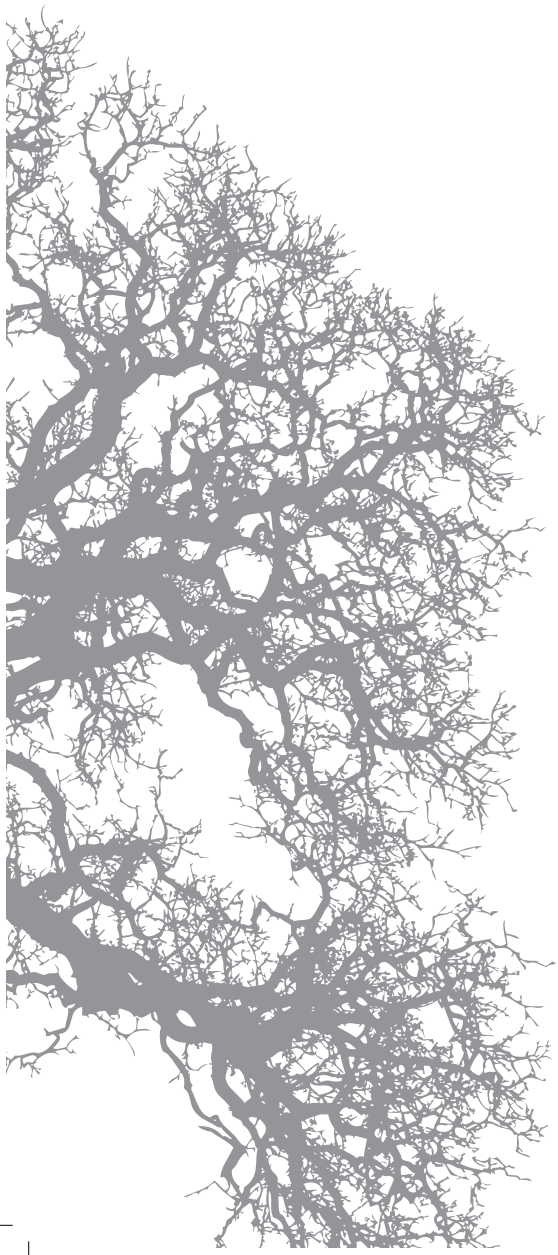
73. Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Niessen WJ, Hofman A, Krestin GP, Breteler MM. Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study. *Neurology* 2008;1:1208-1214.
74. Cordonnier C, Al-Shahi Salman R, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain* 2007;130:1988-2003.
75. Gregoire SM, Chaudhary UJ, Brown MM, Yousry TA, Kallis C, Jager HR, Werring DJ. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. *Neurology* 2009;24:1759-1766.
76. Cordonnier C, Potter GM, Jackson CA, Doubal F, Keir S, Sudlow CL, Wardlaw JM, Al-Shahi Salman R. improving interrater agreement about brain microbleeds: development of the Brain Observer MicroBleed Scale (BOMBS). *Stroke* 2009;40:94-99.
77. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, Launer LJ, Van Buchem MA, Breteler MMB. Cerebral microbleeds: a guide to detection and interpretation. *Lancet Neurol* 2009;8:165-174.
78. Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *Neuroimage* 2003;20:1714-1722.
79. Tournier JD, Mori S, Leemans A. Diffusion tensor imaging and beyond. *Magn Reson Med* 2011;65:1532-1556.
80. Vernooij MW, Ikram MA, Vrooman HA, Wielopolski PA, Krestin GP, Hofman A, Niessen WJ, Van der Lugt A, Breteler MM. White matter microstructural integrity and cognitive function in a general elderly population. *Arch Gen Psychiatry* 2009;66:545-553.
81. Wen W, Zhu W, He Y, Kochan NA, Reppermund S, Slavin MJ, Brodaty H, Crawford J, Xia A, Sachdev P. Discrete neuroanatomical networks are associated with specific cognitive abilities in old age. *J Neurosci* 2011;26:1204-1212.
82. Hsu JL, Chen YL, Leu JG, Jaw FS, Lee CH, Tsai YF, Hsu CJ, Bai CH, Leemans A. Microstructural white matter abnormalities in type 2 diabetes mellitus: a diffusion tensor imaging study. *Neuroimage* 2012;16:1098-1105.
83. Reijmer YD, Brundel M, de Bresser J, Kappelle LJ, Leemans A, Biessels GJ. Microstructural white matter abnormalities and cognitive functioning in type 2 diabetes: a diffusion tensor imaging study. *Diabetes Care* 2013;36:137-144.
84. Yau PL, Javier D, Tsui W, Sweat V, Bruehl H, Borod JC, Convit A. Emotional and neutral declarative memory impairments and associated white matter microstructural abnormalities in adults with type 2 diabetes. *Psychiatry Res* 2009;30:223-230.
85. Yau PL, Javier DC, Ryan CM, Tsui WH, Ardekani BA, Ten S, Convit A. Preliminary evidence for brain complications in obese adolescents with type 2 diabetes mellitus. *Diabetologia* 2010;53:2298-2306.

86. 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* 2013b;62:2112-2115.
87. D'Esposito M, Deouell LY, Gazzaley A. Alterations in the BOLD fMRI signal with ageing and disease: a challenge for neuroimaging. *Nat Rev Neurosci* 2003;4:863-872
88. Musen G, Jacobson AM, Bolo NR, Simonson DC, Shenton ME, McCartney RL, Flores VL, Hoogenboom WS. Resting-state brain functional connectivity is altered in type 2 diabetes. *Diabetes* 2012;61:2375-2379.
89. Xia W, Wang S, Sun Z, Bai F, Zhou Y, Yang Y, Wang P, Huang Y, Yan Y. Altered baseline brain activity in type 2 diabetes: A resting-state fMRI study. *Psychoneuroendocrinology* 2013;38:2493-2501.
90. Zhou H, Lu W, Shi Y, Bai F, Chang J, Yuan Y, Teng G, Zhang Z. Impairments in cognition and resting-state connectivity of the hippocampus in elderly subjects with type 2 diabetes. *Neurosci Lett* 2010;31:5-10.
91. Hoth KF, Gonzales MM, Tarumi T, Miles SC, Tanaka H, Haley AP. Functional MR imaging evidence of altered functional activation in metabolic syndrome. *AJNR Am J Neuroradiol* 2011;32:541-547.
92. Sabri O, Hellwig D, Schreckenberger M, Schneider R, Kaiser HJ, Wagenknecht G, Mull M, Buell U. Influence of diabetes mellitus on regional cerebral glucose metabolism and regional cerebral blood flow. *Nucl Med Commun* 2000;21:19-29.
93. Baker LD, Cross DJ, Minoshima S, Belongia D, Watson GS, Craft S. Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. *Arch Neurol* 2011;68:51-57.
94. Kuczynski B, Jagust W, Chui HC, Reed B. An inverse association of cardiovascular risk and frontal lobe glucose metabolism. *Neurology* 2009;72:738-743.
95. Detre JA, Rao H, Wang DJ, Chen YF, Wang Z. Applications of arterial spin labeled MRI in the brain. *J Magn Reson Imaging* 2012;35:1026-1037.
96. Poels MM, Ikram MA, Vernooij MW, Krestin GP, Hofman A, Niessen WJ, van der Lugt A, Breteler MM. Total cerebral blood flow in relation to cognitive function: The Rotterdam Scan Study. *J Cereb Blood Flow Metab* 2008;28:1652-1655.
97. Rabbitt P, Scott M, Thacker N, Lowe C, Jackson A, Horan M, Pendleton N. Losses in gross brain volume and cerebral blood flow account for age-related differences in speed but not in fluid intelligence. *Neuropsychology* 2006;20:549-557.
98. Fulesdi B, Limburg M, Bereczki D, Kaplar M, Molnar C, Kappelmayer J, Neuwirth G, Csiba L. Cerebrovascular reactivity and reserve capacity in type II diabetes mellitus. *J Diabetes Complic* 1999;13:191-199.
99. Kario K, Ishikawa J, Hoshide S, Matsui Y, Morinari M, Eguchi K, Ishikawa S, Shimada

K. Diabetic brain damage in hypertension: role of renin-angiotensin system. *Hypertension* 2005;45:887-893.

100. Tiehuis AM, Vincken KL, van den Berg E, Hendrikse J, Manschot SM, Mali WP, Kappelle LJ, Biessels GJ. Cerebral perfusion in relation to cognitive function and type 2 diabetes. *Diabetologia* 2008;17:1321-1326.
101. Novak V, Last D, Alsop DC, Abduljalil AM, Hu K, Lepicovsky L, Cavallerano J, Lipsitz LA. Cerebral blood flow velocity and periventricular white matter hyperintensities in type 2 diabetes. *Diabetes Care* 2006;29:529-534.
102. Nagamachi S, Nishikawa T, Ono S, Ageta M, Matsuo T, Jinnouchi S, Hoshi H, Ohnishi T, Futami S, Watanabe K. Regional cerebral blood flow in diabetic patients: evaluation by N-isopropyl-123I-IMP with SPECT. *Nucl Med Commun* 1994;15:455-460.
103. Wakisaka M, Nagamachi S, Inoue K, Morotomi Y, Nunoi K, Fujishima M. Reduced regional cerebral blood flow in aged noninsulin-dependent diabetic patients with no history of cerebrovascular disease: evaluation by N-isopropyl-123I-p-iodoamphetamine with single-photon emission computed tomography. *J Diabet Complications* 1990;4:170-174.
104. Brundel M, van den Berg E, Reijmer YD, de Bresser J, Kappelle LJ, Biessels GJ. Cerebral haemodynamics, cognition and brain volumes in patients with type 2 diabetes. *J Diabetes Complications* 2012;26:205-209.
105. Van Oers CA, Manschot SM, van Huffelen AC, Kappelle LJ, Biessels GJ. Cerebrovascular reserve capacity is preserved in a population-based sample of patients with type 2 diabetes mellitus. *Cerebrovasc Dis* 2006;27:46-50.
106. Kadoi Y, Hinohara H, Kunimoto F, Saito S, Ide M, Hiraoka H, Kawahara F, Goto F. Diabetic patients have an impaired cerebral vasodilatory response to hypercapnia under propofol anesthesia. *Stroke* 2003;34:2399-2403.
107. Hidasi E, Kaplar M, Dioszeghy P, Bereczki D, Csiba L, Limburg M, Fulesdi B. No correlation between impairment of cerebrovascular reserve capacity and electrophysiologically assessed severity of neuropathy in noninsulin-dependent diabetes mellitus. *J Diabetes Complications* 2002;16:228-234.
108. Miller BL. A review of chemical issues in 1H NMR spectroscopy: N-acetyl-L-aspartate, creatine and choline. *NMR Biomed* 1991;4:47-52.
109. Ajilore O, Haroon E, Kumaran S, Darwin C, Binesh N, Mintz J, Miller J, Thomas MA, Kumar A. Measurement of brain metabolites in patients with type 2 diabetes and major depression using proton magnetic resonance spectroscopy. *Neuropsychopharmacology* 2007;32:1224-1231.
110. Geissler A, Frund R, Scholmerich J, Feuerbach S, Zietz B. Alterations of cerebral metabolism in patients with diabetes mellitus studied by proton magnetic resonance spectroscopy. *Exp Clin Endocrinol Diabetes* 2003;111:421-427.

111. Modi S, Bhattacharya M, Sekhri T, Rana P, Tripathi RP, Khushu S. Assessment of the metabolic profile in Type 2 diabetes mellitus and hypothyroidism through proton MR spectroscopy. *Magn Reson Imaging* 2008;26:420-425.
112. Sahin I, Alkan A, Keskin L, Cikim A, Karakas HM, Firat AK, Sagirci A. Evaluation of in vivo cerebral metabolism on proton magnetic resonance spectroscopy in patients with impaired glucose tolerance and type 2 diabetes mellitus. *J Diabetes Complications* 2008;22:254-260.
113. Kreis R, Ross BD. Cerebral metabolic disturbances in patients with subacute and chronic diabetes mellitus: detection with proton MR spectroscopy. *Radiology* 1992;184:123-130.
114. Lin Y, Zhou J, Sha L, Li Y, Qu X, Liu L, Chen H, An Z, Wang Y, Sun C. Metabolite Differences in the Lenticular Nucleus in Type 2 Diabetes Mellitus Shown by Proton MR Spectroscopy. *AJNR Am J Neuroradiol* 2013;34:1693-1696.
115. Tiehuis A, van der Meer F, Mali W, Pleizier M, Biessels GJ, Kappelle LJ, Luijten P. MR spectroscopy of cerebral white matter in type 2 diabetes; no association with clinical variables and cognitive performance. *Neuroradiology* 2010;52:155-161.
116. Brundel M, de Bresser J, van Dillen JJ, Kappelle LJ, Biessels GJ. Cerebral microinfarcts: a systematic review of neuropathological studies. *J Cereb Blood Flow Metab* 2012;32:425-436.
117. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesions. *Lancet Neurol* 2012;11:272-282.
118. Van Veluw SJ, Zwanenburg JJ, Engelen-Lee J, Spliet WG, Hendrikse J, Luijten PR, Biessels GJ. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. *J Cereb Blood Flow Metab* 2013;33:322-329.
119. Hong M, Lee VM. Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons. *J Biol Chem* 1997;1:19547-19553.
120. Edbauer D, Willem M, Lammich S, Steiner H, Haass C. Insulin-degrading enzyme rapidly removes the beta-amyloid precursor protein intracellular domain (AICD). *J Biol Chem* 2002;19:13389-13393.
121. Qiu WQ, Walsh DM, Ye Z, Vekrellis K, Zhang J, Podlisny MB, Rosner MR, Safavi A, Hersh LB, Selkoe D.J. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J Biol Chem* 1998;4:32730-32738.



3 CEREBRAL CORTICAL THICKNESS IN PATIENTS WITH TYPE 2 DIABETES

M Brundel

M van den Heuvel

J de Bresser

LJ Kappelle

GJ Biessels

on behalf of the Utrecht Diabetic Encephalopathy Study Group

Published in: *J. Neurol Sci* 2010;299:126-130.

ABSTRACT

Objective: Type 2 diabetes mellitus (T2DM) is associated with cortical atrophy on MRI. It is unclear whether this atrophy is global or if there are areas with particular vulnerability. We compared regional cortical atrophy between patients with T2DM and controls and examined determinants of atrophy within the T2DM group.

Methods: Cortical surface, volume and thickness were compared between 56 patients with T2DM and 30 controls, both globally and regionally, using the Freesurfer software package. The relationship between atrophy and HbA1c levels, diabetes duration, hypertension, a history of macrovascular disease and cerebral small vessel disease was analyzed within the T2DM group, with linear regression analyses, adjusted for age and gender.

Results: Total cortical surface, total cortical volume and mean cortical thickness for both hemispheres were consistently lower in the T2DM group (between group differences: 0.5 to 4%), but the effects were only significant in the right hemisphere ($p<0.05$). Post-hoc regional analyses revealed significant differences in the hippocampal region (between group differences cortical thickness and volume: 5 to 20.5%) and the middle temporal gyrus (between group differences cortical surface and volume ~8%). Within the T2DM group, smaller cortical thickness of the hippocampal region was associated with cerebral small vessel disease, but no associations between vascular or metabolic determinants and cortical atrophy were found.

Conclusion: The effects of T2DM on cortical grey matter are most pronounced in the temporal lobe. This should be considered when atrophy is used as a marker in etiological or therapeutical studies.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder in the elderly and has been associated with mild to moderate impairments in cognitive functioning[1] and an increased risk of dementia.[2] T2DM is also associated with abnormalities on brain MRI.[3] Lacunar infarcts[3] are commonly found in patients with T2DM and microbleeds may also be more prevalent.[4] Although the association of T2DM with total white matter hyperintensity (WMH) volume was less consistent in early studies,[3] recent studies report a 1.5 to 2-fold increase in WMH volume in T2DM patients[5] and accelerated WMH progression compared to controls.[6]

T2DM is also associated with modest brain atrophy.3 The medial temporal lobe may be particularly vulnerable,[7-9] but it is still unclear if the effects of T2DM on brain volume are global or region specific. There is also uncertainty about risk factors for accelerated atrophy in patients with T2DM.

The present study aimed to assess whether T2DM is associated with global or regional abnormalities in the cerebral cortex, in particular cortical thickness, surface and volume. In addition, the relationship between metabolic and vascular factors and (regional) cortical abnormalities was assessed.

METHODS

Participants

Patients and controls participated in the second survey (2006-2008) of the Utrecht Diabetic Encephalopathy Study, a study on determinants of impaired cognition in T2DM. Detailed descriptions of the study have been published elsewhere.[10,11] At the first survey (2002-2004) patients were recruited through their general practitioner and age, gender and IQ matched controls were recruited among the spouses and acquaintances of the patients. Exclusion criteria for all participants were a psychiatric or neurological disorder (unrelated to diabetes) that could influence cognitive functioning, a history of alcohol or substance abuse or dementia, and a fasting blood glucose ≥7.0 mmol/l for control participants. Individuals with a history of non-invalidating stroke could participate.

A 3D T1-weighted sequence, required for cortical thickness measurements, was included in the MRI scanning-protocol of the second survey and was available from 56 patients and 30 controls. Importantly, these 86 subjects did not differ from the non-participants (n=92) with regard to baseline age or gender ($p>0.05$). Cognitive status,

measured by the Dutch version of the Telephone Interview for Cognitive Status,[12] was verified at the time of the second survey in the majority of non-participants and was similar to the participants (for details see[11]).

The study was approved by the medical ethics committee of the University Medical Center Utrecht and from all participants a written informed consent form was obtained.

Patient characteristics

Participants underwent a standardized interview, physical examination and laboratory tests.[11] The following metabolic and vascular factors were considered as possible determinants of cortical atrophy within the T2DM group: glycosylated haemoglobin (HbA1c) levels (in %), diabetes duration (in years), hypertension (a systolic blood pressure >160 mmHg or diastolic blood pressure >95 mmHg or self-reported use of blood pressure-lowering drugs prescribed primarily for hypertension), a history of macrovascular events (myocardial infarction or stroke requiring hospitalization, or surgical or endovascular treatment for carotid, coronal or peripheral arterial disease) and small vessel disease (the highest tertile of WMH volume (see below), or a lacunar infarct on brain MRI).

Brain MRI

Brain MR images were acquired on a 1.5 Tesla Philips MR system using a standardized protocol, consisting of an axial T1 (repetition time in ms (TR): 234, echo time in ms (TE): 2), inversion recovery (IR) (TR: 2919, TE: 22, inversion time in ms (TI): 410), fluid attenuated inversion recovery (FLAIR) (TR: 6000, TE: 100, TI: 2000) (all 38 contiguous slices, voxel size: 0.9 x 0.9 x 4.0) and a sagittal 3D T1 (TR: 7.0, TE: 3.2; 170 slices, voxel size: 0.94 x 0.94 x 1.00).

Lacunar infarcts were identified by two raters (MB, JB) who were blinded for clinical data. A lesion was considered a lacunar infarct if its core was hypo-intense on T1 and FLAIR, <1.5 cm and if its appearance was unlike a perivascular space. In cases the raters disagreed, consensus was obtained with a third rater (GB).

Image processing

Whole brain cortical thickness was measured at each point of the cortical mantle with the freely available and extensively validated Freesurfer software (available at http://surfer.nmr.mgh.harvard.edu). This included a surface-based method that calculated the distance between the grey and white matter boundary after segmentation of grey matter, white matter and CSF. The cortical surface was parcellated into 34

gyral-based regions in each hemisphere.[13] Mean cortical volume and surface were calculated for each of these regions. In addition, in the left and right hippocampal region cortical thickness and volume was calculated.[14]

Total brain, cortical grey matter, subcortical structures, peripheral cerebrospinal fluid (CSF), lateral ventricular and WMH volume were measured on the IR and FLAIR images by k-Nearest Neighbor-based probabilistic segmentation, an automatic and validated approach to brain segmentation,[15] using a previously published protocol.[16] To correct for between subject differences in intra-cranial volume (volume of all classified tissues combined) all volumes were expressed as a percentage of intra-cranial volume.

Statistical analysis

Between-group differences in patient characteristics were analyzed with independent samples t-tests for means and $\chi 2$ tests for proportions. Linear regression analyses, adjusted for age and gender, were performed to compare brain volumes, total cortical volume, total cortical surface and mean cortical thickness of each hemisphere between the groups. To assess whether there were specific regions of cortical thinning in patients with T2DM compared to controls, each point of the cortical mantle was explored. A map was generated of absolute differences in cortical thickness between controls and diabetic patients. In addition, average cortical volume, surface and thickness were calculated for each of the 34 cortical regions and the hippocampal region. Post-hoc analyses were performed in regions where the age and gender adjusted t-value for the contrast in cortical thickness, volume or surface between the T2DM and control group was at least 3.5 (corresponding with $\alpha<0.001$ for this sample size).

To identify possible determinants of cortical atrophy, linear regression analyses adjusted for age and gender were performed for whole brain measures within the T2DM group. These determinants included HbA1c levels (median split at 7.0%), diabetes duration (median split at 11 years), hypertension, a history of macrovascular events and cerebral small vessel disease. These analyses were repeated in the cortical regions that differed between patients and controls.

RESULTS

Participant characteristics are shown in table 1. Age and gender distribution was similar for the groups, but as expected, the metabolic and vascular risk factor

Table 1 Participant characteristics

	Control participants N = 30	Patients with T2DM N = 56	p-values
Demographics			
Men/women (% men)	13/17 (43)	27/29 (48)	0.821
Age (years)	68.1 ± 4.3	70.0 ± 5.2	0.099
Risk factors for heart and vessel disease			
Mean arterial pressure (mmHg)	101 ± 10	100 ± 11	0.488
Use of antihypertensive drugs (%)	7 (23)	38 (68)	<0.001
Hypertension (%) [a]	10 (33)	42 (75)	<0.001
Hypercholesterolemia (%) [b]	15 (52)	40 (74)	0.040
Smoking ever (%)	21 (70)	36 (64)	0.593
BMI (kg/m2)	26.8 ± 3.9	28.3 ± 4.9	0.162
Any macrovascular event (%) [c]	3 (10)	17 (31)	0.035
Diabetes related factors			
Diabetes duration (years)	-	13.6 ± 6.8	-
HbA1c level (%)	5.7 ± 0.5	7.1 ± 1.0	<0.001
Fasting glucose (mmol/l)	5.6 ± 0.8	8.3 ± 3.0	<0.001
Use of insulin (%)	-	27 (48)	-

[a] Defined as a systolic pressure >160 mm Hg or a diastolic pressure >95 mm Hg or use of antihypertensive drugs primarily for hypertension.

[b] Defined as a fasting cholesterol >6.2 mmol/l or self-reported use of lipid lowering drugs.

[c] Defined as history of a myocardial infarction or stroke requiring hospitalization, or endovascular treatment of carotid, coronal or peripheral arterial disease.

Table 2 brain MRI volumetry

	Control participants (N=30) [a]	Patients with T2DM (N=56) [a]	p-values	Adjusted mean differences [b] (95% CI)
Total brain	78.7 ± 2.1	76.7 ± 2.8	0.002	-1.48 (-2.52; -0.45)
Cortical grey matter	34.1 ± 2.5	32.6 ± 2.6	0.012	-1.22 (-2.30; -0.14)
Subcortical structures	44.2 ± 1.5	43.8 ± 1.5	0.267	-0.26 (-0.94; 0.43)
Peripheral CSF	19.0 ± 1.7	20.5 ± 2.6	0.011	1.14 (0.12; 2.16)
Lateral ventricles	2.2 ± 1.3	2.8 ± 1.1	0.070	0.36 (-0.16; 0.88)
WMH	0.4 ± 0.6	0.4 ± 0.4	0.948	-0.03 (-0.26; 0.19)

[a] Mean relative (expressed as % intracranial volume) brain volumes ± SD. Volume measurements with automated segmentation in controls (n=28) and patients with T2DM (n=50)

[b] Adjusted for age and gender

WMH: white matter hyperintensities

profile of the groups differed. Table 2 shows volumes of total brain, grey and white matter, peripheral CSF, lateral ventricles and WMH. Patients with T2DM had a smaller total brain volume and cortical grey matter volume and larger peripheral CSF volume than controls (between group differences: 1.9 to 6.0%, all $p<0.05$). No significant difference in WMH volume was observed.

Between group analyses on cortical atrophy are presented in table 3. Total cortical surface, total cortical volume and mean cortical thickness for both hemispheres were consistently lower in the T2DM group (between group differences 0.5 to 4%), but only the reduction in cortical surface and volume in the right hemisphere were statistically significant ($p<0.05$). Additional analyses were performed on regional differences in cortical atrophy (figure 1). Between group differences in cortical thickness, surface and volume were not equally distributed across the whole cortex. Differences were most pronounced in the hippocampal region (between group differences in cortical thickness and volume 5 to 20.5%) and the middle temporal gyrus (between group differences cortical surface and volume 6.7 to 8.3%) in both hemispheres.

Table 4 shows the analyses on determinants of cortical atrophy in patients with T2DM None of the metabolic and vascular factors was significantly associated with

Table 3 Cortical atrophy: whole hemisphere and regional between group analyses

	Control participants [a] (N=30)	Patients with T2DM [a] (N=56)
Whole hemisphere analyses		
Total surface L (cm^2)	779.8 ± 67.3	753.0 ± 77.5
Total surface R (cm^2)	786.8 ± 69.5	757.0 ± 77.1
Total volume L (cm^3)	191.4 ± 16.4	184.4 ± 18.7
Total volume R (cm^3)	193.5 ± 16.4	185.5 ± 19.4
Mean thickness L (mm)	2.09 ± 0.08	2.06 ± 0.08
Mean thickness R (mm)	2.09 ± 0.08	2.07 ± 0.08
Post-hoc regional analyses		
Hippocampal region		
Volume L (cm^3)	3.6 ± 0.3	3.3 ± 0.4
Volume R (cm^3)	3.8 ± 0.3	3.5 ± 0.4
Thickness L (mm)	1.32 ± 0.32	1.02 ± 0.25
Thickness R (mm)	1.33 ± 0.44	1.03 ± 0.32
Middle temporal gyrus		
Surface L (cm^2)	32.7 ± 4.0	30.3 ± 4.8
Surface R (cm^2)	34.1 ± 4.4	31.2 ± 4.2
Volume L (cm^3)	9.9 ± 1.3	9.1 ± 1.4
Volume R (cm^3)	10.8 ± 1.2	9.8 ± 1.3
Mean thickness L (mm)	1.72 ± 0.11	1.71 ± 0.18
Mean thickness R (mm)	1.74 ± 0.16	1.72 ± 0.20

L = left; R = right

[a] Mean cortical surface, volume and thickness ± SD

[b] Percentage adjusted mean difference of cortical surface, volume or thickness of control participants

[c] Adjusted for age and gender

* $p < 0.05$

** $p < 0.01$

	Relative differences [b] (%)	Adjusted mean differences [c] (95% CI)	t-value
	3.3	-25.5 (-54.4; 3.4)	-1.753
	3.7	-29.5 (-58.6; -0.3) *	-2.007
	3.5	-6.7 (-13.9; - 0.5)	-1.846
	4.0	-7.7 (-15.0; -0.3) *	-2.062
	1.0	-0.02 (-0.06; 0.02)	-1.035
	0.5	-0.01 (-0.05; 0.02)	-0.662
	5.0	- 0.2 (-0.32; -0.03) *	-2.416
	6.8	-0.26 (-0.41; -0.10) **	-3.260
	20.5	-0.27 (-0.38; -0.15) **	-4.522
	19.5	-0.26 (-0.42; -0.09) **	-3.117
	6.7	-2.2 (-4.0; -0.5) *	-2.517
	8.2	-2.8 (-4.6; -1.1) **	-3.234
	8.1	-0.8 (-1.3; -0.3) **	-2.962
	8.3	-0.9 (-1.4; -0.4) **	-3.702
	0.1	0.00 (-0.07; 0.07)	0.061
	0.2	0.00 (-0.09; -0.08)	-0.090

Table 4 Determinants of cortical atrophy in patients with type 2 diabetes (N=56)

	Total surface (cm^2)	Total volume (cm^3)	Mean thickness (mm)
Higher HbA1c [a]	6.1 (-29.3; 41.5)	5.4 (-3.1; 14.0)	0.03 (-0.01; 0.08)
Longer diabetes duration [a]	7.7 (-29.4; 44.7)	0.4 (-8.7; 9.5)	-0.01 (-0.06; 0.04)
Hypertension [b]	-2.9 (-43.5; 37.7)	0.4 (-9.6; 10.4)	-0.01 (-0.06; 0.04)
Macrovascular event [c]	-11.6 (-50.0; 26.8)	-1.7 (-11.2; 7.8)	-0.01 (-0.06; 0.04)
SVD [d]	11.2 (-24.8; 47.1)	4.1 (-4.7; 12.9)	0.00 (-0.05; 0.04)

Data are age and gender adjusted regression coefficients with 95% CI, measures of both hemispheres were averaged. Negative regression coefficients reflect more atrophy in the subgroup with the determinant. MTG = middle temporal gyrus

[a] HbA1c-levels and diabetes duration were dichotomized at their median values; 7.0 % and 11.0 years, respectively.

[b] Defined as a systolic pressure >160 mm Hg or a diastolic pressure >95 mm Hg or use of antihypertensive drugs primarily for hypertension.

[c] Defined as a myocardial infarction, stroke or endovascular treatment of carotid, coronal or peripheral arterial disease.

[d] SVD = small vessel disease, defined as the highest tertile of WMH volume, or any lacunar infarction.

* $p < 0.01$

Hippocampal region volume (cm^3)	Hippocampal region thickness (mm)	MTG Surface (cm^2)	MTG volume (cm^3)	MTG thickness (mm)
-0.02 (-0.17; 0.20)	-0.01 (-0.14; 0.12)	0.1 (-1.9; 2.0)	0.3 (-0.2; 0.9)	0.07 (-0.02-0.16)
-0.08 (-0.27; 0.11)	-0.04 (-0.17; 0.10)	-0.3 (-2.3; 1.7)	0.0 (-0.5; 0.6)	0.04 (-0.05; 0.14)
-0.07 (-0.28; 0.13)	-0.05 (-0.20; 0.10)	-0.9 (-3.2; 1.3)	-0.3 (-0.9; 0.3)	-0.02 (-0.12; 0.08)
0.03 (-0.17; 0.23)	-0.05 (-0.09; 0.19)	-0.7 (-2.8; 1.4)	-0.1 (-0.7; 0.5)	-0.02 (-0.12; 0.07)
-0.10 (-0.28; 0.08)	-0.17 (-0.29;-0.05) *	0.9 (-1.1; 2.9)	0.2 (-0.3; 0.8)	0.08 (0.00; 0.17)

total brain measurements, or regional atrophy. In the T2DM group, 44.6% of the patients had small vessel disease. Post-hoc regional analyses in the hippocampal region and middle temporal gyrus revealed a smaller cortical thickness of the hippocampal region in patients with small vessel disease ($p<0.01$).

DISCUSSION

We observed a subtle effect of T2DM on whole brain cortical atrophy. Most importantly, this atrophy was not equally distributed across the whole brain. Vulnerable areas were the hippocampal region and the middle temporal gyrus, with a remarkable symmetry in these two regions. Vascular and metabolic factors were not significantly associated with global or regional atrophy in the T2DM group. However, atrophy in the hippocampal region was associated with the presence of small vessel disease.

To the best of our knowledge, there are no previous studies on cortical thickness in patients with T2DM. Previous studies mainly examined whole brain atrophy.[17] A limited number of studies have addressed atrophy in specific brain regions. In line with the present results these studies have suggested the medial temporal lobe to be a vulnerable region.[7-9] Others observed regional effects in the anterior cingulate and orbitofrontal region.[18] Taken together, these studies indicate that despite the fact that T2DM may have an overall effect on brain volume, there also appear to

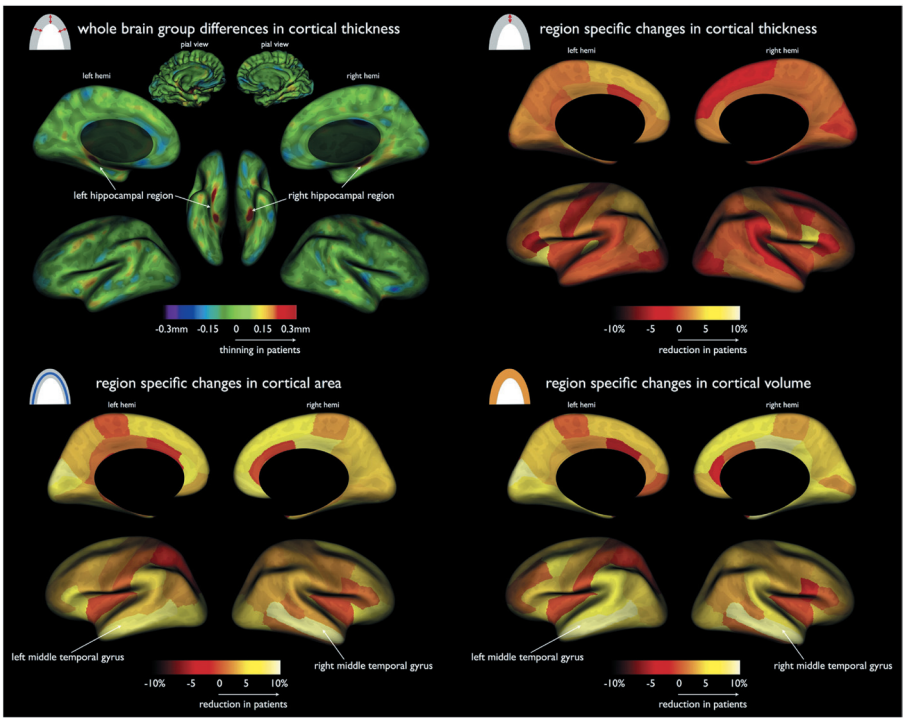


Figure 1 Whole brain group differences in cortical thickness expressed as absolute differences in cortical thickness between controls and patients with T2DM. Region specific changes in cortical thickness, area and volume for the 34 cortical regions of each hemisphere, absolute differences between controls and patients with T2DM.

be region specific effects. Interestingly, temporal lobe atrophy is also a cardinal feature of Alzheimer's disease (AD).[19-20] Previous studies on cortical thickness in patients with (early) AD have reported that reductions in cortical thickness are most pronounced in the medial temporal lobe.[21-23] It should be noted, however, that medial temporal lobe atrophy is not specific to AD-type pathology. In line with the present observations, previous studies have observed an association between the degree of medial temporal lobe atrophy and small vessel disease, both in patients with AD and in the general population.[24-25] Hence, for now, atrophy in this region can only be interpreted as a marker of mixed (i.e. degenerative and vascular) pathology.

Strength of the present study is the detailed image analysis, which now clearly shows the vulnerable cortical areas in T2DM. We applied rigorous statistical correction for repeated testing in the different brain regions. It should be mentioned,

however, that it is difficult to separate grey and white matter in the hippocampal region with the present technique, which may affect the reliability of the cortical thickness and volume measurements in this region. Nevertheless, the symmetry of the atrophy pattern further confirms the robustness of our results. Our method does also not differentiate between different cortical structures in the hippocampal region. The relative modest sample size and the cross-sectional design are other limitations, which may have particularly affected our secondary analyses on vascular and metabolic risk factors of brain atrophy.

In summary, the effects of T2DM on cortical grey matter are most pronounced in the temporal lobe. This should be considered when atrophy is used as a marker in etiological or therapeutical studies.

REFERENCES

1. Allen KV, Frier BM, Strachan MWJ. The relationship between type 2 diabetes and cognitive dysfunction: longitudinal studies and their methodological limitations. *Eur J Pharmacol* 2004;490:169-175.
2. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. *Lancet Neurol* 2006;5:64-74.
3. van Harten B, de Leeuw FE, Weinstein HC, Scheltens P, Biessels GJ. Brain Imaging in Patients With Diabetes: A systematic review. *Diabetes Care* 2006;29:2539-2548.
4. Cordonnier C, Al-Shahi SR, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain* 2007;130:1988-2003.
5. Jongen C, van der GJ, Kappelle LJ, Biessels GJ, Viergever MA, Pluim JP. Automated measurement of brain and white matter lesion volume in type 2 diabetes mellitus. *Diabetologia* 2007;50:1509-1516.
6. Gouw AA, van der Flier WM, Fazekas F, van Straaten EC, Pantoni L, Poggesi A, Inzitair D, Erkinjuntti T, Wahlund LO, Waldemar G, Schmidt R, Scheltens P, Barkhof F; LADIS Study Group. Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. *Stroke* 2008;39:1414-1420.
7. den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MM. Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia* 2003;46:1604-1610.
8. Korf ES, White LR, Scheltens P, Launer LJ. Brain aging in very old men with type 2 diabetes: the Honolulu-Asia Aging Study. *Diabetes Care* 2006;29:2268-2274.
9. Gold SM, Dziobek I, Sweat V, Tirsi A, Rogers K, Bruehl H, Tsui W, RIchardson S, Javier E, Convit A. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. *Diabetologia* 2007;50:711-719.
10. Manschot SM, Brands AM, van der Grond J, Kessels RP, Algra A, Kappelle LJ, Biessels GJ; Utrecht Diabetic Encephalopathy Study Group. Brain magnetic resonance imaging correlates of impaired cognition in patients with type 2 diabetes. *Diabetes* 2006;55:1106-1113.
11. Van den Berg, Reijmer YD, de BJ, Kessels RP, 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.
12. Kempen GI, Meier AJ, Bouwens SF, van DJ, Verhey FR. [The psychometric properties of the Dutch version of the Telephone Interview Cognitive Status (TICS)]. *Tijdschr Gerontol Geriatr* 2007;38:38-45.
13. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS, Killiany RJ. An automated labeling

system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 2006;31:968-980.

14. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montilo A, Makris N, Rosen B, Dale AM. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002;33:341-355.
15. Anbeek P, Vincken KL, van Bochove GS, van Osch MJ, van der GJ. Probabilistic segmentation of brain tissue in MR imaging. *Neuroimage* 2005;27:795-804.
16. De Bresser J, Tiehuis A, van den Berg E., Reijmer YD, Jongen C, Kappelle J, Mali WP, Viergever MA, Biessels GJ; Utrecht Diabetic Encepahlopathy Study Group. Progression of cerebral atrophy and white matter hyperintensities in patients with type 2 diabetes. *Diabetes Care* 2010;33:1309-1314.
17. Jongen C, Biessels GJ. Structural brain imaging in diabetes: a methodological perspective. *Eur J Pharmacol* 2008;585:208-218.
18. Kumar A, Haroon E, Darwin C, Pham D, Ajilore O, Rodriguez G, Mintz J. Gray matter prefrontal changes in type 2 diabetes detected using MRI. *J Magn Reson Imaging* 2008;27:14-19.
19. Juottonen K, Laakso MP, Partanen K, Soininen H. Comparative MR analysis of the entorhinal cortex and hippocampus in diagnosing Alzheimer disease. *AJNR Am J Neuroradiol* 1999;20:139-144.
20. Risacher SL, Saykin AJ, West JD, Shen L, Firpi HA, McDonald BC. Baseline MRI predictors of conversion from MCI to probable AD in the ADNI cohort. *Curr Alzheimer Res* 2009;6:347-361.
21. Simmons A, Westman E, Muehlboeck S, Mecocci P, Vellas B, Tsolaki M, Kloszewska I, Wahlund LO, Soininen H, Lovestone S, Evans A, Spenger C; AddNeuroMed Consortium. MRI measures of Alzheimer's disease and the AddNeuroMed study. *Ann N Y Acad Sci* 2009;1180:47-55.
22. Wang L, Goldstein FC, Veledar E, Levey AI, Lah JJ, Meltzer CC, Holder CA, Mao H. Alterations in cortical thickness and white matter integrity in mild cognitive impairment measured by whole-brain cortical thickness mapping and diffusion tensor imaging. *AJNR Am J Neuroradiol* 2009;30:893-899.
23. Bakkour A, Morris JC, Dickerson BC. The cortical signature of prodromal AD: regional thinning predicts mild AD dementia. *Neurology* 2009;72:1048-1055.
24. De Leeuw FE, Korf E, Barkhof F, Scheltens P. White matter lesions are associated with progression of medial temporal lobe atrophy in Alzheimer disease. *Stroke* 2006;37:2248-2252.
25. Appelman AP, Exalto LG, van der GY, Biessels GJ, Mali WP, Geerlings MI. White matter lesions and brain atrophy: more than shared risk factors? A systematic review. *Cerebrovasc Dis* 2009;28:227-242.

4

CEREBRAL HAEMODYNAMICS, COGNITION AND BRAIN VOLUMES IN PATIENTS WITH TYPE 2 DIABETES

M Brundel

E van den Berg

YD Reijmer

J de Bresser

LJ Kappelle

GJ Biessels

On behalf of the Utrecht Diabetic Encephalopathy Study group

Published in: *J Diabetes Complications* 2012;26:205-209.

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is associated with cognitive impairment and brain abnormalities on MRI. The underlying mechanisms are unclear. We examined the relationship between cerebral haemodynamics (cerebral blood flow (CBF) and cerebrovascular reactivity (CVR)) and cognitive performance and brain volumes in patients with T2DM, at baseline and after four years.

Methods: 114 patients with T2DM, aged 56-80 years, underwent a detailed cognitive assessment and MRI scan. In 68 patients the evaluation was repeated after four years. CBF (two-dimensional flow-encoded phase-contrast MRI) and CVR (carbogen breathing response middle cerebral artery; transcranial Doppler) were measured at baseline. Cognitive performance was expressed as composite z-score and regression based index score. Brain volumes were measured on MRI by automated segmentation. The relationship of haemodynamics with cognition and brain volumes was examined with linear regression analyses adjusted for age, sex and IQ.

Results: Mean CVR was 51.8 ± 18.0% and mean rCBF 53.3 ± 11.3 ml/min/100ml brain tissue. CBF was associated with baseline cognitive performance (standardized regression coefficient β (95%CI): 0.17 (0.00; 0.32) and total brain volume (0.23 (0.05; 0.41)). No correlation was found between CVR and baseline cognitive performance. Neither CBF nor CVR predicted change in cognition (CBF 0.11 (-0.21; 0.44); CVR 0.07 (-0.21; 0.36)) or total brain volume (CBF 0.09 (-0.22; 0.39); CVR 0.13 (-0.13; 0.40)) over four years.

Conclusions: CBF was associated with impaired cognition and total brain volume in cross-sectional analyses, but did not predict changes in cognition or brain volumes over time. Apparently, alterations in cerebral haemodynamics play no major etiological role in cognitive decline or change in brain volumes in non-demented individuals with T2DM.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is associated with cognitive dysfunction and an increased risk of dementia.[1] On brain MRI this is accompanied by modest atrophy and a higher white matter hyperintensity (WMH) load.[1,2] We have previously reported that T2DM-related cognitive changes and cerebral atrophy develop slowly over the course of years, at an average rate that is still within the range of that of normal aging.[3,4] Nevertheless, people with T2DM are overrepresented among those older individuals with accelerated cognitive decline.[5] There is still uncertainty on the etiology, but vascular disease is likely to play a role.[1] Indeed clinically manifest atherosclerosis is associated with cognitive impairment in people with T2DM.[2,6] Moreover, alterations in cerebral haemodynamics might affect the brain, also in people without clinically manifest cerebrovascular disease. In a cross-sectional study, we observed that cerebral blood flow (CBF) was related to cognition, but there were no differences in CBF between controls and patients with T2DM.[7] In the present longitudinal study, we further examined the relationship between cerebral haemodynamics, as reflected by CBF and cerebrovascular reactivity (CVR), and cognitive functioning and brain volumes on MRI in patients with T2DM. This relationship was assessed both at baseline and after four years of follow-up.

METHODS

Participants

Participants were included in the Utrecht Diabetic Encephalopathy Study (UDES), a study on determinants of impaired cognition in patients with T2DM. At baseline (2002-2004), 122 patients with T2DM were recruited through their general practitioners. Furthermore, 56 age, sex and IQ matched controls were recruited among spouses and acquaintances of the patients.[2] At inclusion, all participants were between 56 and 80 years of age, functionally independent and Dutch speaking. Diabetes duration had to be at least one year. Exclusion criteria were a psychiatric or neurological disorder (unrelated to diabetes) that could influence cognitive functioning, a history of alcohol or substance abuse or dementia, and a fasting blood glucose ≥7.0 mmol/l for control participants. Participants with a history of transient ischaemic attacks or non-disabling stroke could be included.

At follow-up four years later (2006-2008), seven participants had died, four could not be contacted and 59 were not willing or able to participate. Reasons for not

participating were: lack of interest (n=28); comorbidity (n=22; three reported dementia (two patients, one control)); and other reasons (n=9). One patient with T2DM was excluded because of severe comorbid disease and one control participant fulfilled the criteria for T2DM and was therefore excluded from the control group. Hence, 106 subjects (68 patients and 38 controls) participated in the follow up examination.[4] Baseline characteristics (demographics, cognitive functioning and brain volumes) in participants (n=106) and non-participants (n=70) at follow-up were similar (for details see [3,4]). From 43 of the 70 non-participants at follow-up who were alive and could be contacted a cognitive screening test was obtained by telephone (the Dutch version of the Telephone Interview for Cognitive Status[8]). Mean performance of non-participants (mean score 35.4 ± 5.2) was similar to the participants (36.5 ± 4.6) at follow-up.[4]

The present study only concerns those patients with T2DM from whom baseline CBF or CVR data were available (n=114). Data from the control group served as reference values.

The study was approved by the medical ethics committee of the University Medical Center Utrecht and all participants signed an informed consent form.

Haemodynamics

Both CBF and CVR were measured at baseline. For CBF measurements a 2D-Phase Contrast MR section was positioned at the level of the skull base to measure volume flow in the internal carotid arteries and basilar artery (TR 16 ms; TE 9 ms; flip angle 7.5°; voxel size 0.98x0.98x5.00 mm^3, averages 8; velocity sensitivity 100 cm/s).[9] Total CBF was defined as the sum of flow in both internal carotid arteries and basilar artery. Because total CBF is related to brain size, we calculated relative total CBF (rCBF), expressed as ml/min per 100 ml brain tissue.[10]

CVR was assessed with transcranial Doppler (TCD) as described previously.11 CVR in response to a raise in CO_2 was determined as the relative change in blood flow velocity in both middle cerebral arteries after 1.5 minute of carbogen inhalation. Left and right CVR were averaged.

Neuropsychological examination

Neuropsychological examination consisted of 11 tasks, covering 5 cognitive domains (i.e. attention and executive functions, information processing speed, memory, abstract reasoning, and visuoconstruction).[4] A division in these cognitive domains was made a priori, according to standard neuropsychological practise and cognitive theory.[12] For the present study we used a composite z-score of tests addressing the

first three domains, because these are particularly sensitive to the effects of T2DM and vascular disease.[4] These domains were assessed with the following tests: 1) attention and executive functions: Trail-making Test (Part B), Stroop Color-Word Test (Part 3), Brixton Spatial Anticipation Test, a letter fluency test using the 'N' and 'A' and category fluency (animal naming); 2) information processing speed: Trail-making Test (Part A), Stroop Color-Word Test (Part 1 and 2), subtest Digit Symbol of the Wechsler Adult Intelligence Scale 3rd edition (WAIS-3); 3) memory: forward and backward digit span of the WAIS-3, Corsi Block-tapping Task, Rey Auditory Verbal Learning Test, Location Learning Test, delayed trial of the modified Taylor Complex Figure.[13]

Raw test scores at baseline and follow-up were standardized into z-scores per test, by using the pooled mean of baseline scores of the whole group. The z-scores of each domain were calculated by averaging the test scores comprising that domain; these z-scores were averaged to obtain one composite z-score. In secondary analyses, each cognitive domain was addressed separately.

Change in cognitive performance over time was expressed as a regression based index (RBI) score, using the control group as a reference, taking age, sex and estimated IQ into account.[14] A negative RBI score reflects greater cognitive decline than expected from a control participant with a similar demographic profile and cognitive performance at baseline. The RBI score is preferred over using change in z-scores over time, because it reduces confounding by learning effects and regression to the mean.[14] Mean change in performance across the three domains was expressed as a composite RBI score. In secondary analyses, the RBI score of each cognitive domain was addressed separately. Pre-morbid IQ was assessed by the Dutch version of the National Adult Reading test.[13]

Brain volume measurements

MRI scans were acquired on a 1.5 Tesla Philips MR scanner using a standardized protocol (38 contiguous slices, voxel size: 0.9 x 0.9 x 4.0 mm^3) and consisted of an axial T1 (repetition time in ms (TR)/echo time in ms (TE): 234/2), T2 (TR/TE: 2200/100), proton density (TR/TE: 2200/11), inversion recovery (IR) (TR/TE/inversion time in ms (TI): 2919/22/410) and fluid attenuated inversion recovery (FLAIR) (TR/TE/TI: 6000/100/2000).[3]

After registration of all sequences to the FLAIR image and an inhomogeneity correction, a baseline brain mask was created by a k-means clustering algorithm with 8 clusters for every patient using all sequences. The baseline FLAIR image was rigidly registered to the follow-up FLAIR image within patients. The resulting

transform parameters were applied to the baseline mask to create follow-up masks. The uncorrected FLAIR images were multiplied voxelwise times the mask images followed by an inhomogeneity correction. The IR and FLAIR images were used for a k-nearest neighbour-based probabilistic segmentation algorithm that measured total brain, lateral ventricular and WMH volumes on both time points.[3,15] Volumes were expressed as percentage of total intra-cranial volume to correct for between subject differences in head size. Volume changes between baseline and follow-up scans were calculated within participants.

Markers of atherosclerosis

At baseline, carotid intima-media thickness (IMT) was measured in both carotid arteries in a 1-cm trajectory proximal to the beginning of the dilatation of the carotid bulb in 3 different longitudinal projections. IMT was calculated as the mean of these 6 measurements as described previously.[16]

A history of cardiovascular events was defined as a clinical history of myocardial infarction, stroke (not including TIA) or endovascular or surgical treatment of carotid, coronal or peripheral arterial disease.

Statistical analysis

Baseline WMH volume was multiplied by 100 and natural-log-transformed because of non-normal distribution (Kolmogorov-Smirnov, $p<0.05$). The relationship between baseline markers of haemodynamics (rCBF, CVR) and baseline or change over time of cognition and brain volumes was examined within the T2DM group with linear regression analyses adjusted for age and sex (cognition also for IQ). These relationships were expressed as standardized betas.

RESULTS

Baseline characteristics of the patients with T2DM are presented in table 1. Mean CVR was 51.8 ± 18.0% and mean rCBF 53.3 ± 11.3 ml/min per 100 ml brain tissue (for the control participants: mean CVR 46.2 ± 16.6% and mean rCBF 57.7 ± 12.2 ml/min per 100 ml brain tissue). At baseline, the patient group with T2DM had a worse cognitive performance than the control group (adjusted mean difference in composite z-score between the T2DM and control group (95%CI) -0.24 (-0.43; -0.05) (For details see [2]). Moreover, patients with T2DM had a significantly smaller relative total relative brain volume (-1.36% of intracranial volume (-2.31; -0.40)) and

Table 1 Baseline characteristics

Patients with type 2 diabetes (n = 114)	
Sex (men)	59 (52)
Age (years)	65.9 ± 5.8
Estimated IQ_	98 ± 15
Diabetes duration (years)	8.7 ± 6.1
HbA1c level (%)	6.8 ± 1.1
Fasting glucose levels (mmol/L)	8.6 ± 2.9
Use of insulin	32 (28)
Smoking (present or past)	78 (68)
Hypertension [a]	62 (54)
Hypercholesterolemia [b]	69 (61)
Cardiovascular event [c]	30 (26)
Stroke	7 (6)
Carotid intima-media thickness (mm) (n = 108)	0.93 ± 0.17
Cerebrovascular reactivity (%) (n = 96)	51.8 ± 18.0
Total relative cerebral blood flow (ml/min per 100ml brain tissue) (n = 85) [d]	53.3 ± 11.3

Data shown are means ± SD or n. Values in parenthesis are percentages.

[a] Defined as a systolic blood pressure >160 mmHg or a diastolic pressure >95 mmHg or use of antihypertensive drugs primarily for hypertension

[b] Defined as a fasting cholesterol >6.2 mmol/l or self-reported use of lipid lowering drugs

[c] Defined as history of a myocardial infarction, stroke (not including TIA) or endovascular treatment of carotid, coronal or peripheral arterial disease.

[d] Defined as the summed flow in both carotid arteries and the basilar artery

Table 2 Relationship between baseline haemodynamics in patients with T2DM and cognition at baseline and change in cognition during follow-up

	Composite cognition
Baseline [a]	
CVR (%) (n=96)	0.04 (-0.16; 0.25)
rCBF (ml min^{-1} $100ml^{-1}$) (n=85)	0.17 (0.00; 0.32) *
Longitudinal change [b]	
CVR (%) (n=58)	0.07 (-0.21; 0.36)
rCBF (ml min^{-1} $100ml^{-1}$) (n=47)	0.11 (-0.21; 0.44)

Data are standardized regression Beta-coefficients (95% CI), adjusted for age, sex and IQ

CVR = cerebrovascular reactivity, rCBF = relative cerebral blood flow

[a] At baseline cognition is expressed as a (composite) z-score

[b] The change in cognition over time is expressed as regression based index (RBI) score

* $p < 0.05$

Table 3 Relationship between baseline haemodynamics in patients with T2DM and brain volumes at baseline and change in brain volumes during follow up

	Total brain volume (%ICV)
Baseline	
CVR (%) (n=96)	-0.06 (-0.27; 0.13)
rCBF (ml min^{-1} $100ml^{-1}$) (n=85)	0.23 (0.05; 0.41) *
Longitudinal change	
CVR (%) (n=58)	0.13 (-0.13; 0.40)
rCBF (ml min^{-1} $100ml^{-1}$) (n=47)	0.09 (-0.22; 0.39)

Data are standardized regression Beta-coefficients (95% CI), adjusted for age and sex

WMH = white matter hyperintensities, CVR = cerebrovascular reactivity, rCBF = relative cerebral blood flow, %ICV: percentage of intracranial volume

[a] Relative baseline WMH volumes were multiplied by 100 and natural-log-transformed.

* $p < 0.05$

Attention and executive functioning	Information processing speed	Memory
0.02 (-0.12; 0.16)	0.08 (-0.13; 0.30)	-0.03 (-0.15; 0.10)
0.19 (0.02; 0.38) *	0.21 (0.01; 0.42) *	-0.10 (-0.29; 0.10)
0.17 (-0.10; 0.44)	0.03 (-0.22; 0.28)	-0.16 (-0.43; 0.10)
0.16 (-0.16; 0.47)	0.01 (-0.19; 0.20)	0.00 (-0.48; 0.48)

Lateral ventricular volume (%ICV)	WMH volume [a]
0.20 (-0.03; 0.41)	0.05 (-0.19; 0.26)
-0.12 (-0.33; 0.11)	-0.18 (-0.44; 0.00)
0.05 (-0.14; 0.19)	0.14 (-0.14; 0.56)
-0.08 (-0.53; 0.38)	-0.23 (-0.62; 0.08)

larger lateral ventricular volume (0.37% (-0.12; 0.87) and WMH volume (0.35 (-0.13; 0.83), WMH volumes were multiplied by 100 and natural-log-transformed) (For details see [3]).

Tables 2 and 3 show the associations of haemodynamics with cognition and brain volumes. At baseline, lower rCBF was associated with a lower composite z-score (β (95%CI): 0.17 (0.00; 0.32), p=0.046). In secondary analyses on each cognitive domain separately, lower rCBF was significantly associated with the domains attention and executive functioning (0.19 (0.02; 0.38), p=0.034) and information processing speed (0.21 (0.01; 0.42), p=0.039). After additional adjustment for a history of stroke, the association between rCBF and composite z-score was attenuated (0.16 (0.00; 0.34), p=0.053). In contrast, adjustment for C-IMT (0.17 (0.00; 0.33), p=0.056), cardiovascular events (0.19 (0.02; 0.33), p=0.025) or both (0.19 (0.02; 0.33), p=0.027) did not essentially change the association. Lower rCBF was also significantly associated with lower baseline relative total brain volume (0.23 (0.05; 0.41, p=0.01). Adjustment for C-IMT (0.24 (0.05; 0.42), p=0.01), cardiovascular events (0.24 (0.07; 0.42) p=0.01), a clinical history of stroke (0.22 (0.05; 0.39), p=0.01) or all these factors (0.25 (0.06; 0.43) did not change this association. Importantly, in the patients with T2DM who also attended the follow up examination, the same relationship at baseline between CBF and composite cognition (0.25 (0.02; 0.45) and total relative brain volume (0.27 (-0.02; 0.58) was found. It should be noted that rCBF was expressed in ml/min per 100 ml brain tissue. Hence, in patients with relatively lower brain volume, perfusion per 100 ml brain tissue was even less.

No significant relationships between CVR and baseline composite z-score, relative total relative brain volume, lateral ventricular volume and WMH volume were observed.

Over four years, across the group of patients with T2DM cognitive decline was not accelerated compared to the reference group (composite score: RBI score ± SD -0.06 ± 0.73; mean change in z-score ± SD -0.11 ± 0.24; for details see [4]). Within the T2DM patient group neither baseline CVR, nor rCBF, was significantly related to cognitive decline over the 4 years of the study (table 2).

Over the four years, total relative brain volume decreased (change over time (%) ± SD: -1.46 ± 0.71) and lateral ventricular (0.36% ± 0.25) and WMH volume increased (0.14% ± 0.18) in the patients with T2DM. Only the increase of lateral ventricular volume was significantly accelerated relative to controls (adjusted difference between T2DM and control group (95%CI): 0.11% (0.00; 0.22)) (for details see [3]) No significant associations between baseline rCBF or CVR and change of brain volumes over four years were found (table 3).

DISCUSSION

In this study sample of non-demented older patients with T2DM, rCBF was associated with impaired cognition and total brain volume in cross-sectional analyses. However, cerebral haemodynamics at baseline appeared to be no predictor for changes in cognition or brain volumes over time.

There is still uncertainty on the risk factors for cognitive decline and brain abnormalities in T2DM. Previous studies, mostly cross-sectional in design, point to chronic hyperglycaemia and microvascular complications as well as atherosclerosis as relevant factors.[1] A recent study found a relationship between atherosclerosis (ankle-brachial index) and incident dementia after six years in older patients with T2DM.[6] Likewise, in the general population, atherosclerosis, also in blood vessels not supplying the brain, is associated with cognitive impairments.[17-19] In previous papers on the present study population, we reported that in patients with T2DM a history of cardiovascular events was associated with impaired information processing speed and memory, and more severe vascular lesions on MRI,[2] but cardiovascular events did not predict cognitive decline over four years,[4] or brain volume changes.[3]

The association between atherosclerosis and cognitive dysfunction and dementia might be mediated by alterations in cerebral haemodynamics. However, inter-individual variation in haemodynamics can obviously also reflect processes other than atherosclerosis. Indeed, in the present study the relationship between rCBF and cognitive dysfunction at baseline was independent of IMT and a history of cardiovascular events. However, this association was not independent of a clinical history of stroke. To the best of our knowledge there are no other published studies on the relation between cerebral haemodynamics and cognition in patients with T2DM. In the general population, rCBF was found to be cross-sectionally associated with cognitive functioning (especially information processing speed and executive functioning). This association was mediated by brain atrophy[20] and WMH.[21] Furthermore, earlier cross-sectional studies found cerebral hypoperfusion to be associated with cognitive impairment and Alzheimer's disease.[22]

Regarding the relationship between cerebral haemodynamics and structural brain MRI markers, previous cross-sectional studies, not specifically addressing T2DM, found reduced rCBF in non-demented older individuals and older people with vascular risk factors to be associated with WMH severity.[23,24] However, previous studies on the relation between rCBF and brain atrophy in elderly individuals with vascular risk factors show conflicting results. While one study observed no relationship between rCBF and atrophy,[24] another study found rCBF to be associated

with subcortical atrophy.10 In patients with T2DM, a cross-sectional relationship between lower regional rCBF and brain atrophy has previously been reported,[25] in line with the cross-sectional analyses in the present dataset.

Remarkably, cerebral haemodynamics were unrelated to cognitive decline and progression of atrophy in this sample. This does not support a causal role for disturbed haemodynamics in cognitive dysfunction and atrophy as might be inferred from the cross-sectional studies. Reverse causality might even play a role. In other words, the demand for CBF may be relatively lower in abnormal atrophic brain tissue, thus giving rise to the observed associations in the cross-sectional studies. This does not imply, however, that the impact of T2DM on the brain is independent of vascular disease. In fact, white matter hyperintensities and lacunar infarcts on brain MRI are more common in patients with T2DM and these lesions are associated with cognitive decline and brain atrophy.[26] Moreover, recent autopsy studies indicate that vascular brain damage is the key neuropathological determinant of increased dementia risk in T2DM.[27]

Strengths of the present study are the prospective design in combination with detailed assessment of cognitive status and precise brain volume measurements. A limitation is that the sample size was modest. Nevertheless, point estimates for the regression coefficients in the longitudinal data were close to 0, with relatively narrow 95% confidence intervals, indicating that variation in haemodynamics could explain, at most, 5% of the variation (coefficient of determination) in the changes in cognition and brain volumes over four years, and that our negative results are not due to lack of statistical power. Another limitation could be the possible selective loss to follow-up. However, participants and nonparticipants were comparable at baseline (demographics, brain volumes) and follow-up (cognitive status).[3,4] Finally, our global measures of CBF and CVR may have missed regional abnormalities.

In summary, the present study showed that CBF was associated with impaired cognition and total brain volume in cross-sectional analyses but neither CBF, nor CVR predicted cognitive decline or change of brain volumes over time. Apparently alterations in cerebral haemodynamics play no major etiological role in cognitive decline or change in brain volumes in non-demented individuals with T2DM.

REFERENCES

1. Biessels GJ, Deary IJ, Ryan CM. Cognition and diabetes: a lifespan perspective. *Lancet Neurol* 2008;7:184-190.
2. Manschot SM, Biessels GJ, de Valk HW, Algra A, Rutten GE, 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: 2388-2397.
3. De Bresser J, Tiehuis AM, van den Berg E, Reijmer YD, Jongen C, Kappelle LJ, Mali WP, Viergever MA, Biessels GJ. Progression of cerebral atrophy and white matter hyperintensities in patients with type 2 diabetes. *Diabetes Care* 2010;33:1309-1314.
4. Van den Berg E, Reijmer YD, de Bresser J, Kessels RP, 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.
5. Reijmer YD, van den Berg E, Ruis C, Kappelle LJ, Biessels GJ. Cognitive dysfunction in patients with type 2 diabetes. *Diabetes Metab Res Rev* 2010;26:507-519.
6. Bruce DG, Davis WA, Casey GP, Starkstein SE, Clarnette RM, Foster JK, Almeida OP, Davis TM. Predictors of cognitive impairment and dementia in older people with diabetes. *Diabetologia* 2008;51:241-248.
7. Tiehuis AM, Vincken KL, van den Berg E, Hendrikse J, Manschot SM, Mali WP, Kappelle LJ, Biessels GJ. Cerebral perfusion in relation to cognitive function and type 2 diabetes. *Diabetologia* 2008;51:1321-1326.
8. Kempen GI, Meier AJ, Bouwens SF, van Deursen J, Verhey FR . [The psychometric properties of the Dutch version of the Telephone Interview Cognitive Status (TICS)]. *Tijdschr Gerontol Geriatr* 2007;38:38-45.
9. Spilt A, Box FM, van der Geest RJ, Reiber JH, Kunz P, Kamper AM, Blauw GJ, van Buchem MA. Reproducibility of total cerebral blood flow measurements using phase contrast magnetic resonance imaging. *J Magn Reson Imaging*, 2002;16:1-5.
10. Appelman AP, van der Graaf Y, Vincken KL, Tiehuis AM, Witkamp TD, Mali WP, Geerlings MI . Total cerebral blood flow, white matter lesions and brain atrophy: the SMART-MR study. *J Cereb Blood Flow Metab* 2008;28:633-639.
11. Van Oers CA, Manschot SM, van Huffelen AC, Kappelle LJ, Biessels GJ. Cerebrovascular reserve capacity is preserved in a population-based sample of patients with type 2 diabetes mellitus. *Cerebrovasc Dis* 2006;22:46-50.
12. Lezak MD, Howieson DB, Loring DW. Neuropsychological Assessment. New York, NY: Oxford Press 2004.
13. Manschot SM, Brands AM, van der Grond J, Kessels RP, Algra A, Kappelle LJ, Biessels

GJ. Brain magnetic resonance imaging correlates of impaired cognition in patients with type 2 diabetes. *Diabetes* 2006;55:1106-1113.

14. Temkin NR, Heaton RK, Grant I, Dikmen SS. Detecting significant change in neuropsychological test performance: a comparison of four models. *J Int Neuropsychol Soc* 1999;5:357-369.
15. Anbeek P, Vincken KL, van Bochove GS, van Osch MJ, van der Grond J. Probabilistic segmentation of brain tissue in MR imaging. *Neuroimage* 2005;27:795-804.
16. Olijhoek JK, van der Graaf Y, Banga JD, Algra A, Rabelink TJ, Visseren FL. The metabolic syndrome is associated with advanced vascular damage in patients with coronary heart disease, stroke, peripheral arterial disease or abdominal aortic aneurysm. *Eur Heart J* 2004;25:342-348.
17. De la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. *Lancet Neurol* 2004;3:184-190.
18. Hofman A, Ott A, Breteler MM, Bots ML, Slooter AJ, Van Harskamp F, van Duijn CN, Van Broeckhoven C, Grobbee DE . Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. *Lancet* 1997;349:151-154.
19. Mathiesen EB, Waterloo K, Joakimsen O, Bakke SJ, Jacobsen EA, Bonaa KH. Reduced neuropsychological test performance in asymptomatic carotid stenosis: The Tromso Study. *Neurology* 2004;62:695-701.
20. Poels MM, Ikram MA, Vernooij MW, Krestin GP, Hofman A, Niessen WJ, van der Lugt A, Breteler MM. Total cerebral blood flow in relation to cognitive function: The Rotterdam Scan Study. *J Cereb Blood Flow Metab* 2008;28:1652–1655
21. Appelman AP, van der Graaf Y, Vincken KL, Mali WP, Geerlings MI. Combined effect of cerebral hypoperfusion and white matter lesions on executive functioning - The SMART-MR study. *Dement Geriatr Cogn Disord* 2010;29:240-247.
22. Johnson NA, Jahng GH, Weiner MW, Miller BL, Chui HC, Jagust WJ, Gorno-Tempini ML, Schuff N. Pattern of cerebral hypoperfusion in Alzheimer disease and mild cognitive impairment measured with arterial spin-labeling MR imaging: initial experience. *Radiology* 2005;234:851-859.
23. Bastos-Leite AJ, Kuijer JP, Rombouts SA, Sanz-Arigita E, van Straaten EC, Gouw AA, van der Flier WM, Scheltens P, Barkhof F. Cerebral Blood Flow by Using Pulsed Arterial Spin-Labeling in Elderly Subjects with White Matter Hyperintensities. *AJNR Am J Neuroradio* 2008;29:1296-1301.
24. Van Es AC, van der Grond J, ten D, V, de Craen AJ, Blauw GJ, Westendorp RG, dmiraal-Behloul F, van Buchem MA. Associations between total cerebral blood flow and age related changes of the brain. *PLoS ONE* 2010;5:e9825.
25. Last D, Alsop DC, Abduljalil AM, Marquis RP, de BC, Hu K, Cavallerano J, Novak

V. Global and regional effects of type 2 diabetes on brain tissue volumes and cerebral vasoreactivity. *Diabetes Care* 2007;30:1193-1199.

26. Sima AA. Encephalopathies: the emerging diabetic complications. *Acta Diabetologia* 2010;47:279-293.
27. Ahtiluoto S, Polvikoski T, Peltonen M, Solomon A, Tuomilehto J, Winblad B, Sulkava R, Kivipelto M. Diabetes, Alzheimer disease, and vascular dementia: a population-based neuropathologic study. *Neurology* 2010; 75:1195-1202.

5

MICROVASCULAR LESIONS ON ULTRA HIGH-RESOLUTION 7T MRI IN PATIENTS WITH TYPE 2 DIABETES

M Brundel

YD Reijmer

SJ van Veluw

HJ Kuijf

PR Luijten

LJ Kappelle

GJ Biessels

On behalf of the Utrecht Vascular Cognitive Impairment (VCI) Study Group

Published in: *Diabetes* 2014 [Epub ahead of print]

ABSTRACT

Cerebral small vessel disease, including microvascular lesions, is considered to play an important role in the development of type 2 diabetes mellitus (T2DM) associated cognitive deficits. With ultra-high field MRI microvascular lesions (e.g. microinfarcts and microbleeds) can now be visualized *in vivo*. For the present study, 48 nondemented older individuals with T2DM (mean age 70.3 ± 4.1 years) and 49 age-, sex-, and education-matched control subjects underwent a 7 Tesla brain MRI scan and a detailed cognitive assessment. The occurrence of cortical microinfarcts and cerebral microbleeds was assessed on FLAIR and T1-weighted images and T2*-weighted images respectively, compared between the groups and related to cognitive performance. Microinfarcts were found in 38% of controls and 48% of patients with T2DM. Microbleeds were present in 41% of control participants, and 33% of patients (all $p>0.05$). Presence and number of microinfarcts or microbleeds were unrelated to cognitive performance. This first study on microvascular brain lesions in people with T2DM at ultra-high field MRI showed no increased burden of cerebral microvascular damage compared to controls.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is associated with cognitive dysfunction and a twofold increased risk of dementia.[1] The aetiology is incompletely known, but vascular disease is likely to play a role.[1] In the general population vascular disease, in particular cerebral small vessel disease (SVD), is a major contributor to ageing related cognitive decline and dementia.[2] On conventional magnetic resonance imaging (MRI) SVD can be visualized as white matter hyperintensities (WMH), lacunar infarcts, and microbleeds.[3] However, these conventional markers of SVD do not capture the full burden of cerebral microvascular damage. Neuropathological studies have identified microinfarcts as another common microvascular pathology that is linked to ante-mortem cognitive decline and dementia.[4]

T2DM is a known risk factor for vascular disease, affecting both large and small vessels. Microvascular complications of T2DM appear in the retina, peripheral nervous system, kidney, and probably also the brain. Ultra-high resolution MRI now, for the first time, permits visualization of cortical microinfarcts *in vivo*[5] and also greatly enhances the detection of cerebral microbleeds.[6] We hypothesized that microinfarcts and microbleeds are more common in patients with T2DM than in controls and that these lesions are associated with cognitive dysfunction. The present study investigated the presence of cortical microinfarcts and cerebral microbleeds with 7 Tesla (7T) in patients with T2DM and in age-matched non-diabetic controls, and explored the relationship between these microvascular lesions and cognitive performance.

METHODS

Study population

Patients were recruited through six general practitioners as part of the second Utrecht Diabetic Encephalopathy Study (UDES2).[7] Eight hundred sixty-four randomly selected persons between 65 and 80 years of age (416 patients and 453 controls) received a letter to which they could respond if they were willing to participate. Two hundred sixty-three persons responded that they refused to participate; 168 responded that they were willing to participate, of which 63 patients with T2DM and 61 age-, sex- and education-matched controls met our inclusion criteria.

For inclusion, participants had to be 65-80 years of age, functionally independent and Dutch speaking. The diagnosis of diabetes had to be established at least one

year prior to the study. Controls had to have a fasting blood glucose <7.0 mmol/l. Exclusion criteria were contraindications for 7T MRI, a psychiatric or neurological disorder that could influence cognitive functioning (including dementia), recent non-disabling stroke (<2 years) or any disabling stroke, major depression or alcohol abuse. All subjects underwent a standardized evaluation, including medical history, physical and neurological examination, neuropsychological assessment, laboratory testing and both a 3T and 7T MRI, all on the same day.

In 26 participants (15 T2DM, 11 controls) no complete 7T MRI could be obtained due to patient related factors (e.g. contra-indications for 7T MRI or claustrophobia) or technical issues. One control participant proved to have a neurological disease that was not detected upon initial screening, leaving 97 subjects (48 T2DM, 49 controls) for the present study.

The study was approved by the medical ethics committee of the University Medical Center Utrecht and all subjects gave written informed consent.

Medical history and biometric measurements

Medical history and medication use were assessed with standardized questionnaires. Blood pressure was measured at three different time points during the day and averaged. BMI was calculated as weight in kilograms divided by the square of height in meters. Fasting glucose, HbA1c and cholesterol levels were measured with standard laboratory testing. Impaired fasting glucose (IFG) was defined as fasting glucose levels of 5.6-6.9 mmol/L, according to the ADA criteria.

Data on microvascular complications were recorded. Retinopathy was defined as self-report of a physician diagnosis. Neuropathy was rated with the Toronto Clinical Neuropathy Scoring System,[8] but without a sensory test for temperature, so that the maximum score is 18 points. A score >6 was considered as indicative of neuropathy. A patient was considered to have macroalbuminuria in case of an albumin-to-creatinine ratio of >300 µg/mg (according to ADA criteria), based on laboratory testing of a first midstream urine sample in the morning.

A macrovascular event was defined as a clinical history of myocardial infarction, stroke (not including TIA) or endovascular or surgical treatment of carotid, coronal or peripheral arterial disease.

MRI scanning protocol

Scans were acquired on a 7T MR system (Philips Healthcare, Cleveland, OH, USA) with a volume transmit and 16 or 32-channel receive head coil (Nova Medical, Wilmington, MA). The standardized protocol included a dual-echo gradient echo

sequence (repetition time (TR)/echo time (TE) = 20/6.9;15.8 ms, reconstructed voxel size 0.39 x 0.39 x 0.35 mm3); a volumetric (3D) T1-weighted sequence (TR/inversion time (TI)/TE 4.8/1240/2.2 ms, reconstructed voxel size 0.66 x 0.66 x 0.50 mm^3); and a 3D fluid-attenuated inversion recovery (FLAIR) sequence (TR/TI/TE: 8000/2325/300 ms, reconstructed voxel size 0.49 x 0.49 x 0.40 mm^3).

Scans on the 3T MR system (Philips Medical Systems, Best, the Netherlands) were acquired with a standardized protocol including, among others, a FLAIR sequence (TR/TI/TE 11000/2800/125 ms, reconstructed voxel size 0.96 x 0.95 x 3 mm^3), a 3D T1-weighted sequence (TR/TI/TE 7.9/955/4.5 ms, voxel size 1.00 x 1.00 x 1.00 mm^3) and a dual-echo T2-weighted sequence (TR/TE 3198/19;140 ms, reconstructed voxel size 0.96 x 0.95 x 3.00 mm^3). 3T MRI data were used for the detection of brain infarcts and the determination of brain volumes and WMH volumes.

Detection of microvascular lesions

Microvascular lesions were rated visually on 7T MRI scans by two independent raters, blinded to diabetes status and clinical information. In case of disagreement, consensus was obtained in a consensus meeting.

Cortical microinfarcts were defined as either small hyperintense (probably gliotic) lesions or hypointense with a hyperintense rim (probably cystic) lesions on the FLAIR image, corresponding with a hypointense lesion on the T1-weighted sequence (as previously described,[9] but without use of a T2-weighted sequence) (Fig. 1). Each lesion had to be detectable on sagittal, coronal, and transversal views, ≤3mm in length and restricted to the cortex to be classified as a microinfarct. Due to low signal-to-noise ratio on FLAIR images in the temporal lobes and cerebellum, these areas were not investigated.

Microbleeds were detected by the previously described semi-automatic method based on the radial symmetry transform (RST).[10] A slightly modified adaptation to the method was made by incorporating minimum intensity projection images. This improves the sensitivity and reduces the number of suspected microbleed locations. The RST result was then censored visually to select true microbleeds.
The inter-rater agreement was good for number of microbleeds (ICC (95% CI): 0.83 (0.75; 0.88)) and moderate for number of microinfarcts (ICC=0.39 (0.21;0.55)).

Other MRI measures

WMHs, cerebral infarcts and brain volumes were determined on 3T MRI scans. WMHs were outlined manually on the FLAIR images and their volumes were calculated. Brain infarcts were rated visually on FLAIR and T1-weighted images

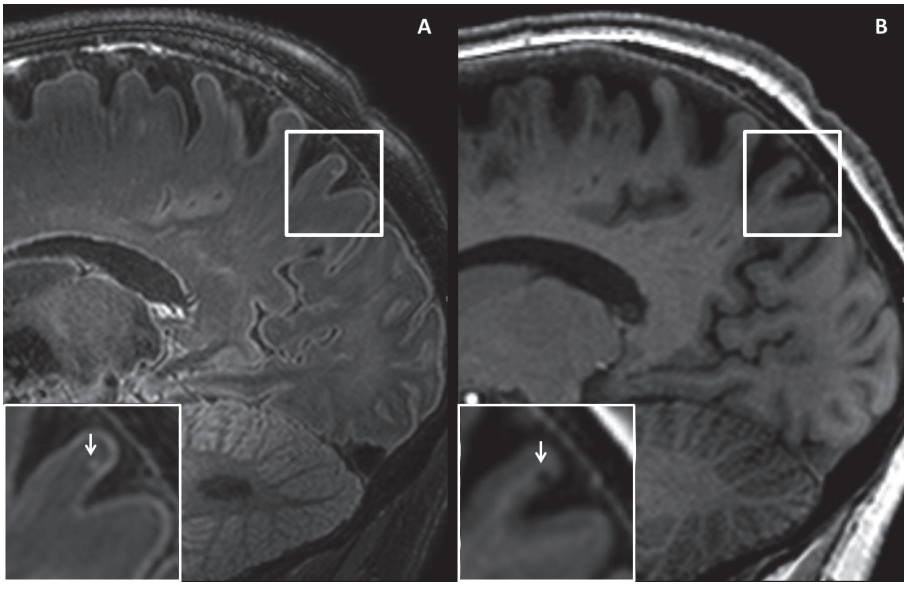


Figure 1 An example of a cortical microinfarct, which appears hyperintense on FLAIR (A) and hypointense on T1-weighted (B) 7 tesla MR images.

and classified as large vessel infarcts (>1.5 cm) or lacunar infarcts. Gray and white matter volumes were computed automatically on the T1-weighted image using the Freesurfer software (http://surfer.nmr.mgh.harvard.edu). Volumes were expressed as a percentage of intracranial volume, which was outlined manually on the T2-weighted images.

Cognitive testing

All participants underwent a detailed standardized cognitive assessment as described earlier,7 including tests for memory, information-processing speed and attention and executive function. For each cognitive test, raw test scores were standardized into z-scores and averaged to obtain one composite z-score per cognitive domain.

IQ was estimated with the Dutch version of the National Adult Reading Test, which is generally accepted to reflect the premorbid level of intellectual functioning.

Statistical analyses

Between-group differences in subject characteristics were analysed with an independent- samples t-test for continuous variables, χ^2 tests for proportions and Mann-Whitney U tests for non-parametric data. Relationships between the presence

of microvascular lesions and cognition were examined with linear regression analyses, adjusted for age, sex, estimated IQ, and group (control or T2DM). Because the numbers of microbleeds and microinfarcts showed a skewed distribution, three groups with 0, 1 or ≥1 lesion were distinguished.

RESULTS

Subject characteristics are summarized in Table 1. Controls and patients with T2DM did not differ in age, sex, and estimated IQ. Patients with T2DM used significantly more often antihypertensive and cholesterol lowering drugs and had a higher BMI than controls. Patients with T2DM performed slightly worse than controls on all three cognitive domains (mean differences in standardized z-scores (95% CI) between patients and controls for information-processing speed: -0.24 (-0.58; 0.11); attention and executive functioning: -0.21 (-0.50; 0.09); memory -0.14 (-0.44; 0.17), all p>0.05), but differences were not statistically significant.

MRI findings are described in Table 2. Cortical microinfarcts were found in 19 (38%) controls (5 subjects showed >1 microinfarcts) and in 23 (48%) patients with T2DM (7 subjects showed >1 microinfarcts), p>0.05 (Fig. 2). Microbleeds were present in 20 (41%) controls (10 subjects showed >1 microbleeds), and in 16 (33%) patients with T2DM (10 patients showed >1 microbleeds), p>0.05 (Fig. 2). Microbleed distribution (i.e. presence and number of lobar and deep/infratentorial microbleeds) was also similar between the groups.

Cerebral gray matter volumes were smaller and lateral ventricle volumes larger in the patients than in the controls, but WMH and white matter volumes and occurrence of brain infarcts did not differ between the groups (Table 2).

Across the two groups, cognitive performance on the three cognitive domains was not related to microvascular lesion load (Regression coefficient B (95% CI) information processing speed: microinfarcts 0.06 (-0.17; 0.29), microbleeds 0.11 (-0.08; 0.31); attention and executive functioning: microinfarcts 0.04 (-0.16; 0.23), microbleeds 0.02 (-0.14; 0.19); and memory: microinfarcts 0.10 (-0.11; 0.31), microbleeds 0.06 (-0.12; 0.24), all p>0.05).

When control participants with IFG (n=25; 51%) were excluded, microbleeds were present in 11 (46%) and microinfarcts in 10 (42%) of the remaining 24 controls. Between group comparisons on MRI markers and cognition for controls without IFG and patients with T2DM did not show results that were different from the main analyses (data not shown).

Table 1 Group characteristics

	Control participants n=49	Patients with T2DM n=48	*p*-value
Age (years)	71.1 ± 4.5	70.3 ± 4.1	0.36
Sex (% male)	30 (61)	26 (54)	0.48
Estimated IQ [a]	103 ± 13	101 ± 12	0.29
Systolic blood pressure (mmHg) [b]	146 ± 22	148 ± 13	0.69
Diastolic blood pressure (mmHg) [b]	81 ± 9	80 ± 10	0.78
Antihypertensive medication (%)	25 (51)	36 (75)	0.02
Total cholesterol (mmol/L)	5.4 ± 1.0	4.7 ± 0.9	<0.01
Cholesterol lowering drugs (%)	21 (43)	34 (71)	<0.01
Current smoking	8 (16)	5 (10)	0.62
BMI (kg/m^2)	26.1 ± 3.2	29.5 ± 5.1	<0.01
Antithrombotic use (%)	12 (25)	18 (38)	0.17
Fasting glucose (mmol/L)	5.6 ± 0.7	8.0 ± 2.0	<0.01
HbA1c (%)	5.7 ± 0.4	6.8 ± 0.8	<0.01
Diabetes duration (years)		11.0 ± 9.3	
Insulin/oral medication or diet (%)		15/33 (31/69)	
Macrovascular event (%) [c]	3 (6)	7 (15)	0.17
Retinopathy (%)	0 (0)	5 (10)	0.02
Peripheral neuropathy (%) [d]	5 (10)	10 (21)	0.15
Macroalbuminuria [e]	0 (0)	1 (2)	0.31
Cognitive performance [f]			
Information-processing speed	0.13 ± 0.57	-0.11 ± 1.07	0.18
Attention and executive functioning	0.09 ± 0.56	-0.11 ± 0.84	0.16
Memory	0.07 ± 0.73	-0.07 ± 0.76	0.37

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

[a] Estimated by the Dutch version of the National Adult Reading Test.

[b] Mean values for three measurements; for one control subject blood pressure was not examined.

[c] Defined as a clinical history of myocardial infarction, stroke (not including TIA) or endovascular or surgical treatment of carotid, coronal or peripheral arterial disease.

[d] Rated with the Toronto Clinical Neuropathy Scoring System[8]

[e] Defined as an albumin-to-creatinine ratio of >300 µg/mg

[f] Data are presented as mean standardized z-scores ± SD.

Table 2 MRI findings

	Control participants n=49	Patients with T2DM n=48	*p*-value
Microvascular lesions [a]			
Cortical microinfarct presence (%)	19 (38)	23 (48)	0.36
Cortical microinfarct No.	0 (0-11)	0 (0-5)	0.35
Microbleed presence (%)	20 (41)	16 (33)	0.33
Microbleed No.	0 (0-5)	0 (0-13)	0.55
Strictly deep/infratentorial microbleeds (%)	3 (7)	1 (2)	0.32
Strictly lobar microbleeds (%)	11 (24)	12 (27)	0.76
Other MRI markers [b]			
White matter hyperintensity volume (mL)	6.9 ± 10.2	5.4 ± 5.5	0.36
Lacunar infarction (%)	13 (27)	10 (21)	0.51
Large vessel infarction (%)	2 (4)	3 (6)	0.63
Gray matter volume (% ICV)	39.1 ± 2.0	38.0 ± 2.2	0.02
White matter volume (% ICV)	30.5 ± 3.1	29.9 ± 2.4	0.30
Lateral ventricle volume (% ICV)	2.0 ± 1.0	2.7 ± 1.5	0.02

Data are presented as mean ± SD, n (%), or median (range). ICV = intracranial volume

[a] Determined at 7 Tesla field strength

[b] Determined at 3 Tesla field strength

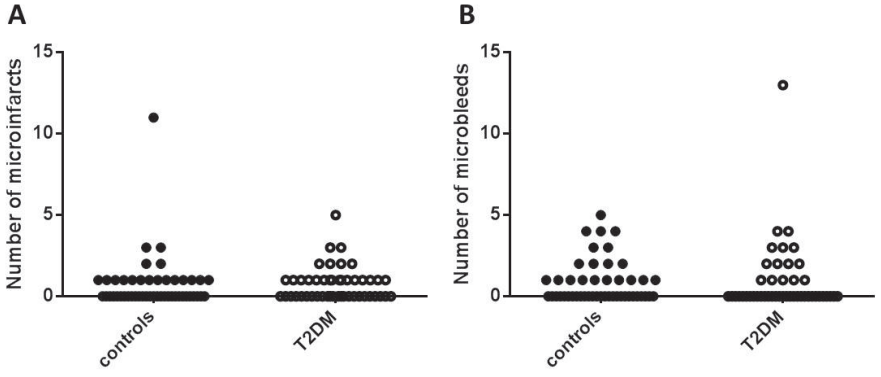


Figure 2 Number of microinfarcts (A) and microbleeds (B) in controls and patients with T2DM. The number of microvascular lesions did not differ between the groups (Mann Whitney U test for number of microinfarcts: p=0.35; for number of microbleeds: p=0.55).

DISCUSSION

In this first study on microvascular brain lesions in people with T2DM at ultra-high field MRI we did not observe an increased occurrence of microinfarcts or microbleeds compared to controls. Presence and number of microinfarcts or microbleeds were unrelated to cognitive performance.

Previous estimates on the occurrence of cerebral microinfarcts are solely based on autopsy studies, which identified them in around 24% of nondemented older individuals, with no significant differences between patients with T2DM and controls.11 However, in autopsy studies that specifically addressed people with dementia, microinfarcts were more common in patients with T2DM than in those without.[12,13] In autopsy studies not specifically focusing on T2DM, microinfarcts have been related to cognitive impairment and dementia diagnosis before death.[14,15] These results suggest that the relationship between microinfarcts and cognition only becomes evident in cognitively symptomatic individuals. This may explain why we did not find a relation between microinfarcts and cognition in the present study.

Studies on the prevalence of microbleeds specifically in patients with T2DM are scarce. A few population-based studies showed diabetes mellitus to be associated with microbleeds (OR 2.2, 95% CI 1.2-4.2).[16] Two recent studies, including the AGES-Reykjavik study, specifically addressed patients with T2DM. They showed no difference in overall microbleed occurrence between patients with T2DM and controls.[17,18] Although, an increased likelihood of ≥2 microbleeds in diabetic patients (7%) compared to controls (4%) was reported.18 Both studies used 1.5T MRI and have found much lower prevalences compared with our study. Microbleeds did not mediate the association of diabetes and worse cognitive performance.[18] In previous large population-based cohorts, not specifically addressing T2DM, only weak associations between microbleeds and cognitive deficits have been reported,[19,20] for example 0.4 MMSE points, for people with ≥5 microbleeds relative to those without.[20] These studies are in line with our results showing modest or no relationships with cognition, despite the high sensitivity of high field strength MRI to detect microbleeds.

Strengths of our study include the advanced technique for the detection of microvascular lesions, with a substantial sample size for an ultra-high field MRI study. We also employed a comprehensive cognitive examination in a well-defined population-based cohort. Importantly, our scan protocol has the advantage over neuropathology that almost the complete supratentorial part of the brain can be assessed (except for the temporal lobes), while autopsy studies only sample a small

portion of the brain microscopically. Moreover, in previous neuropathological studies, the temporal lobe has not been reported as a location of preference.[11] There are also limitations. Our MRI protocol can only detect the largest microinfarcts.[9] It is uncertain if detected lesions are a good representation of the complete lesion load in the brain. Moreover, particularly the assessment of microinfarcts is rater dependent. Nevertheless, because the raters were blinded we do not expect systematic errors in lesion counts for the groups. Finally, in the patient group glycaemia and vascular risk factors were relatively well controlled. Although this does reflect current clinical practice guidelines[21] it may have attenuated the contrast between the groups and may limit generalizability of our findings. Moreover, controls with IFG were not excluded from the study which might add to this effect. Our findings may therefore underestimate the occurrence of microvascular lesions in less well controlled patient populations. Nevertheless, the patients in our cohort did have other brain MRI abnormalities that are typical for T2DM. They had a modest degree of brain atrophy, in line with the literature,[22] and we previously reported that these patients had disturbed white matter connectivity relative to controls,[7,23] despite the observation that WMH volumes and the occurrence of brain infarcts did not differ between the groups, which has also been reported before.[24,25] Finally, the effect size of the difference in cognitive performance compared with controls of around 0.2, although not statistically significant, is in line with a recent meta-analysis of the literature, which reports effect sizes of 0.2-0.4.[26]

To conclude, this study showed no increased burden of cerebral microvascular damage in this well-controlled group of patients with T2DM compared to controls. Further studies should assess whether cerebral microvascular lesions do occur in patients who are less well controlled or have a high burden of microvascular complications elsewhere in the body.

REFERENCES

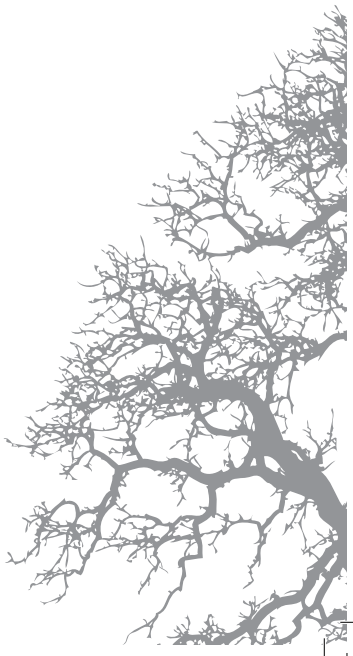
1. Biessels GJ, Deary IJ, Ryan CM. Cognition and diabetes: a lifespan perspective. *Lancet Neurol* 2008;7:184-190.
2. Gorelick PB, Scuteri A, Black SE, DeCarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, Petersen RC, Schneider JA, Tzourio C, Arnett DK, Bennett DA, Chui HC, Higashida RT, Lindquist R, Nilsson PM, Roman GC, Sellke FW, Seshadri S; American Heart Association Stroke Council, Council on Epidemiology and Prevention, Council on Cardiovascular Nursing, Council on Cardiovascular Radiology and Intervention, and Council on Cardiovascular Surgery and Anesthesia. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke* 2011;42:2672-2713.
3. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, Lindley RI, O'Brien JT, Barkhof F, Benavente OR, Black SE, Brayne C, Breteler M, Chabriat H, Decarli C, de Leeuw FE, Doubal F, Duering M, Fox NC, Greenberg S, Hachinski V, Kilimann I, Mok V, Van Oostenbrugge R, Pantoni L, Speck O, Stephan BC, Teipel S, Viswanathan A, Werring D, Chen C, Smith C, van Buchem M, Norrving B, Gorelick PB, Dichgans M; STandards for ReportIng Vascular changes on nEuroimaging (STRIVE v1). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* 2013;12:822-838.
4. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesions. *Lancet Neurol* 2012;11:272-282.
5. Van Veluw SJ, Zwanenburg JJ, Engelen-Lee J, Spliet WG, Hendrikse J, Luijten PR, Biessels, GJ. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. *J Cereb Blood Flow Metab* 2013;33:322-329.
6. Conijn MM, Geerlings MI, Biessels GJ, Takahara T, Witkamp TD, Zwanenburg JJ, Luijten PR, Hendrikse J. Cerebral microbleeds on MR imaging: comparison between 1.5 and 7T. *AJNR Am J Neuroradiol* 2011;32:1043-1049.
7. Reijmer YD, Brundel M, de Bresser J, Kappelle LJ, Leemans A, Biessels GJ. Microstructural white matter abnormalities and cognitive functioning in type 2 diabetes: a diffusion tensor imaging study. *Diabetes Care* 2013;36:137-144.
8. Bril V, Perkins BA. Validations of the Toronto Clinical Scoring System for diabetic polyneuropathy. *Diabetes Care* 2002;25:2048-2052.
9. Van Veluw SJ, Heringa SM, Kuijf HJ, Koek HL, Luijten PR, Biessels GJ. Cerebral Cortical Microinfarcts at 7Tesla MRI in Patients with Early Alzheimer's Disease. *J Alzheimers Dis* 2014;39:163-167.

10. Kuijf HJ, de Bresser J, Geerlings MI, Conijn MM, Viergever MA, Biessels GJ, Vincken KL. Efficient detection of cerebral microbleeds on 7.0 T MR images using the radial symmetry transform. *Neuroimage* 2012;59:2266-2273.
11. Brundel M, de Bresser J, van Dillen JJ, Kappelle LJ, Biessels GJ. Cerebral microinfarcts: a systematic review of neuropathological studies. *J Cereb Blood Flow Metab* 2012;32:425-436.
12. Sonnen JA, Larson EB, Brickell K, Crane PK, Woltjer R, Montine TJ, Craft S. Different Patterns of Cerebral Injury in Dementia With or Without Diabetes. *Arch Neurol* 2009;66:315-322.
13. Alafuzoff I, Aho L, Helisalmi S, Mannermaa A, Soininen H. Beta-amyloid deposition in brains of subjects with diabetes. *Neuropathol Appl Neurobiol* 2009;35:60-68.
14. Launer LJ, Hughes TM, White LR. Microinfarcts, brain atrophy, and cognitive function: the Honolulu Asia Aging Study Autopsy Study. *Ann Neurol* 2011;70:774-780.
15. Arvanitakis Z, Leurgans SE, Barnes LL, Bennett DA, Schneider JA. Microinfarct pathology, dementia, and cognitive systems. *Stroke* 2011;42:722-727.
16. Cordonnier C, Al-Shahi Salman R, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain* 2007;130:1988-2003.
17. Moran C, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Munch G, Wood AG, Forbes J, Greenaway TM, Pearson S, Srikanth V. Brain Atrophy in Type 2 Diabetes: Regional distribution and influence on cognition. *Diabetes Care* 2013;36:4036-4042.
18. Qiu C, Sigurdsson S, Zhang Q, Jonsdottir MK, Kjartansson O, Eiriksdottir G, Garcia ME, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Diabetes, markers of brain pathology, and cognitive cognition: The AGES-Reykjavik study. *Ann Neurol* 2014;75:138-146.
19. Qiu C, Cotch MF, Sigurdsson S, Jonsson PV, Jonsdottir MK, Sveinbjrnsdottir S, Eiriksdottir G, Klein R, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. *Neurology* 2010;75:2221-2228.
20. Poels MM, Ikram MA, van der Lugt A, Hofman A, Niessen WJ, Krestin GP, Breteler MM, Vernooij MW. Cerebral microbleeds are associated with worse cognitive function: The Rotterdam Scan Study. *Neurology* 2012;78:326-333.
21. Buse JB, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, Fonseca V, Gerstein HC, Grundy S, Nesto RW, Pignone MP, Plutzky J, Porte D, Redberg R, Stitzel KF, Stone NJ; American Heart Association; American Diabetes Association. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Circulation* 2007;115:114-126.

22. Espeland MA, Bryan RN, Goveas JS, Robinson JG, Siddiqui MS, Liu S, Hogan PE, Casanova R, Coker LH, Yaffe K, Masaki K, Rossom R, Resnick SM; WHIMS-MRI Study Group. Influence of type 2 diabetes on brain volumes and changes in brain volumes: results from the Women's Health Initiative Magnetic Resonance Imaging studies. *Diabetes Care* 2013;36:90-97.
23. 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;62:2112-2115.
24. Van Elderen SG, de Roos A, de Craen AJ, Westendorp RG, Blauw GJ, Jukema JW, Bollen EL, Middelkoop HA, van Buchem MA, van der Grond J. Progression of brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. *Neurology* 2010;75:997-1002.
25. Schmidt R, Launer LJ, Nilsson LG, Pajak A, Sans S, Berger K, Breteler MM, de Ridder M, Dufouil C, Fuhrer R, Giampaoli S, Hofman A. CASCADE Consortium. Magnetic resonance imaging of the brain in diabetes: the Cardiovascular Determinants of Dementia (CASCADE) Study. *Diabetes* 2004;53:687-692.
26. Palta P, Schneider AL, Biessels GJ, Touradji P , Hill-Briggs F. Magnitude of cognitive dysfunction in adults with type 2 diabetes: a meta-analysis of six cognitive domains and the most frequently reported neuropsychological tests within domains. *J Int Neuropsychol Soc* 2014;20:1-14.

PART II

CEREBAL MICROVASCULAR LESIONS



6 CEREBRAL MICROINFARCTS: A SYSTEMATIC REVIEW OF NEUROPATHOLOGICAL STUDIES

M Brundel

J de Bresser

J van Dillen

LJ Kappelle

GJ Biessels

Published in: *J Cereb Blood Flow Metab* 2012;32:425-436.

ABSTRACT

Vascular cognitive impairment (VCI) is an umbrella term for cognitive dysfunction associated with and presumed to be caused by vascular brain damage. Autopsy studies have identified microinfarcts as an important neuropathological correlate of VCI that escapes detection by conventional MRI. As a frame of reference for future high-resolution MRI studies, we systematically reviewed the literature on neuropathological studies on cerebral microinfarcts in the context of vascular disease, vascular risk factors and cognitive decline and dementia. We identified 32 original patient studies involving 10515 people. The overall picture is that microinfarcts are common, particularly in patients with vascular dementia (weighted average 62%), Alzheimer's disease (43%) and demented patients with both Alzheimer-type and cerebrovascular pathology (33%) compared to non-demented older individuals (24%). In many patients multiple microinfarcts were detected. Microinfarcts are described as minute foci with neuronal loss, gliosis, pallor or more cystic lesions. They are found in all brain regions, possibly more so in the cerebral cortex, particularly in watershed areas. Reported sizes vary from 50 μm to a few mm, which is within the detection limit of current high resolution MRI. Detection of these lesions in vivo would have a high potential for future pathophysiological studies in VCI.

INTRODUCTION

Cerebrovascular disease is a common cause of cognitive decline and dementia. Cognitive decline associated with and presumed to be caused by cerebrovascular disease is referred to as Vascular Cognitive Impairment (VCI).[1] VCI is thus an umbrella term for various clinical syndromes (e.g. vascular dementia, post-stroke dementia) and dementia associated with vascular risk factors. It comprises heterogenic pathological processes that affect both the brain vessels and parenchyma. Damage to brain parenchyma is visible on brain Magnetic Resonance Imaging (MRI) as lacunar and non-lacunar infarcts, macro- and microhaemorrhages and white matter hyperintensities.[2]

Recent brain autopsy studies showed that microinfarcts are another type of vascular brain damage that is related to impaired cognition, also in patients with a clinical diagnosis of Alzheimer's disease (AD) without apparent macroscopic vascular pathology.[3-5] Because of their limited size, microinfarcts go undetected on conventional MRI. However, with the introduction of ultra-high field strength clinical MR scanners, which offer a higher spatial resolution, it may be possible to detect them in vivo. In the light of these new developments, it is important to collect the data that is already available on microinfarcts from neuropathological studies, to form a frame of reference for future MRI studies. The aim of this paper is to systematically review the literature on neuropathological studies on microinfarcts as a marker of brain injury related to vascular diseases, vascular risk factors and cognitive impairment.

MATERIAL AND METHODS

Literature search strategy

Studies were identified by a systematic search of MEDLINE and EMBASE (1966 to July 11th 2011). In brief, we used the search term 'microinfarct' and synonyms, e.g. microinfarction, microischemia, microvascular infarct and microvascular ischemia (for search string see supplementary table 1). Because we expected a limited number of relevant studies, we used a broad array of search terms in full and truncated form to identify all available papers. The bibliographies of relevant original and review articles were screened. Relevant studies were then selected by screening of title and abstract against predefined in- and exclusion criteria. Inclusion criteria were: 1) original patient studies (no reviews) concerning cerebral microinfarcts, either in the general

population or related to vascular brain damage (e.g. stroke, small vessel disease), vascular risk factors (hypertension, diabetes mellitus), or cognitive impairment or dementia; 2) the characteristics, frequency or distribution of microinfarcts had to be described in the study. No restriction on design or pathological assessment method was made. Case reports, narrative reviews, letters, animal studies and articles in languages other than English were excluded. Moreover, studies regarding auto-immune brain diseases (e.g. systemic lupus erythematosus, vasculitis) or acute encephalopathy (e.g. hypertensive or post-anoxic encephalopathy) were excluded. Studies that did not distinguish between microinfarcts and lacunar infarcts (i.e. studies that combined all small infarcts <1.5 cm in their reports) were also excluded.

Data extraction

The following data were extracted from included papers: 1) Study design: pro/retrospective cohort, population-based or hospital-based; 2) Diagnosis of the examined group and sample size per diagnosis; 3) Diagnostic procedure: primarily on clinical criteria, primarily on neuropathological criteria, or on both; 4) Demographics: mean age at death and gender; 5) Pathological assessment method: sampling sites and slice thickness (see supplementary table 2), staining methods and immunohistochemistry. To facilitate interpretation of data presented in tables, data are grouped according to the following diagnoses: reference groups, AD, vascular dementia (VaD), cerebrovascular disease, and mixed dementia groups. Within these groups, the frequency, size, characteristics and distribution of microinfarcts and the number of microinfarcts per patient were listed (table 2).

RESULTS

The literature search of MEDLINE and EMBASE yielded 571 articles. Of these, 516 articles were excluded for the following reasons: reports on microinfarcts in other organs than the brain (n=276), reports on conditions other than chronic cerebrovascular disease, e.g. acute encephalopathy, infection or epilepsy (n=119), animal studies (n=45), narrative reviews (n=33), case reports (n=27), letters/comments (n=5) or language other than English (n=11). Eight more articles were included after screening the reference lists of the included articles. The majority of these were not identified by the original search because cerebral vascular injury was described in more general terms in title and abstract.

Thirty-nine articles met the inclusion criteria. One paper concerned 3 different cohorts,[6] from which we only used the data of the National Alzheimer's Coordinating

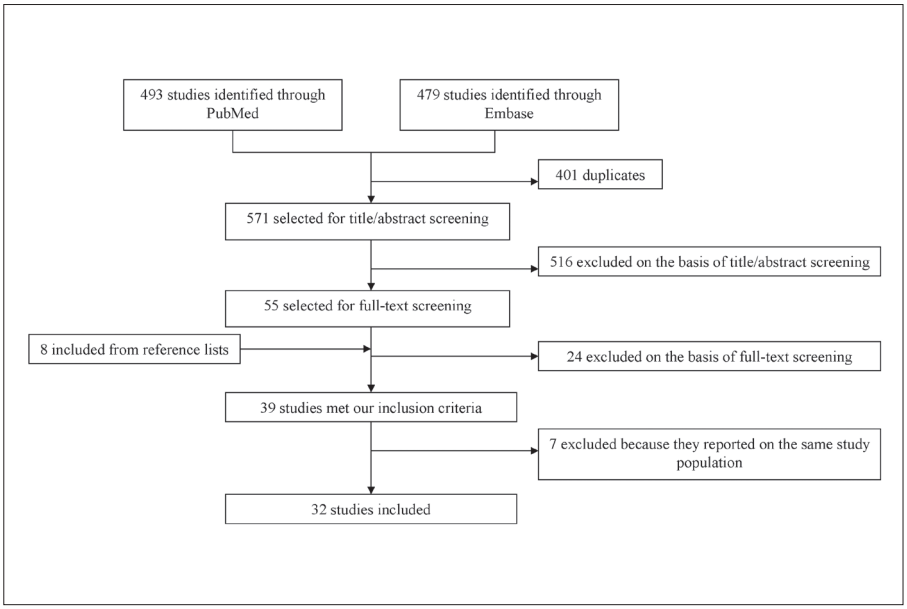


Figure 1 Selection of included studies

Center (NACC). For the other two cohorts in that paper (i.e. the Adult Changes in Thought (ACT) Study and the Honolulu Asia Aging Study (HAAS)) data were extracted from the original published articles.[3,7,8] When more than one paper reported on the same population,[5,7,9–18] the paper with the largest population sample and the most detailed information on microinfarcts was included. There were 2 exceptions. Two out of four papers concerning the ACT cohort[7,9–11] were included, because they described different samples from this cohort.[7,9] Three out of eight papers concerning a cohort of autopsied patients at the Geriatric and Psychiatric Hospitals of the University of Geneva were included.[5,12–18] The paper by Gold et al.[16] described the largest sample. Two additional papers[5,18] were also included because they provided relevant detailed information on sub-samples of this cohort (AD Braak stage III and dementia respectively). Hence, a total of 32 articles were included (Figure 1).

The design of these studies and the population characteristics are summarized in table 1; data on the frequency, size and characteristics of microinfarcts in table 2 and 3.

Characteristics of the included studies

Most studies had a retrospective design (some of a prospectively selected cohort); only four studies were prospective.[3,8,19,20] The greater part (63%) concerned hospital-based cohorts, mostly patients admitted to geriatric hospitals or dementia clinics. Twelve studies (41%) described a population-based cohort,[3,7–9,19,21–25,35,41,50] mostly of

Table 1. Characteristics of included studies

Study	Design [a]		Diagnosis (sample size) [b]
Arvanitakis [19]	P	Population-based	1. Dementia (192) 2. No dementia (233)
Ballard [20]	R	Population-based	1. VaD (19) 2. NDD + VaD (11) 3. AD (37)
Brayne [22]	RP	Population-based	1. Dementia (113) 2. No dementia (100)
De Reuck [40]	RP	Hospital-based	1. AD (45) 2. DLB (8) 3. FTD (12) 4. No dementia (10)
De Reuck [29]	RP	Hospital-based	1. AD – CAA (12) 2. AD – no CAA (28)
Erkinjuntti [33]	RP	Hospital-based	1. AD (5) 2. VaD (27) 3. Dementia due to other causes (5)
Esiri [26]	RP	Hospital-based	1.CVD (19) 2. VaD (24) 3. No dementia (18)
Ghebremedhin [34]	R	Hospital-based	1. DLB (13) 2. PD (102) 3. No dementia, no PD (53)
Gold [16]	RP	Hospital-based	Dementia and no dementia (156)
Haglund [42]	RP	Hospital-based	VaD without AD (26)
Isojima [39]	R	x	1. DLB (25) 2. AD (63)
Kövari [5]	RP	Hospital-based	AD Braak stage 3 (43)
Lee [20]	P	Hospital-based	1. AD + CVD (201) 2. AD (36)
Longstreth [6]	R	Hospital-based	admitted to NACC (6189)
Okamoto [44]	R	Hospital-based	1. AD (8) 2. VaD (6)
Olichney [28]	R	Hospital-based	1. AD – severe CAA (50) 2. AD – mild CAA (198)
Rossi [41]	RP	Hospital- and population-based	1. AD (49) 2. Non-AD dementia (17) 3. MCI (3) 4. No dementia (18)

Diagnosis based on [c]	Mean age at death (range)	Male (%)
Clinical (NINCDS-ADRDA)	1. 89 2. 85	1. 35 2. 43
Clinical (DSM), neuropathology (NINDS-AIREN)	77 (onset dementia)	57
Clinical (DSM)	91 (81-101)	30
Neuropathology (Braak)	1. 81 2. 78 3. 69 4. 69	1. 51 2. 63 3. 50 4. 60
Neuropathology (Braak)	1. 79 2. 76	1. 50 2. 43
Clinical (DSM)	1. 74 (57-83) 2. 80 (61-92) 3. 79 (74-83)	1. 60 2. 37 3. 40
Neuropathology	1. 83 2. 76 3. 80	1. 63 2. 38 3. 55
Clinical	1. 76 2. 75 3. 74	1. 38 2. 59 3. 58
Clinical (CDR)	87 (73-101)	41
Neuropathology	82	50
Neuropathology (AD: CERAD)	1. 81 (67-94) 2. 83 (67-94)	1. 48 2. 35
Neuropathology (Braak)	(73-101)	28
Neuropathology (NIA), clinical (DSM)	1. 79 2. 84	1. 30 2. 48
Neuropathology	81	48
Neuropathology (CERAD, Braak)	1. 79 2. 77	x
Neuropathology (CERAD)	x	1. 66 2. 55
Neuropathology	73 (44-99)	48

Table 1. Characteristics of included studies (extended)

Study	Design[a]		Diagnosis (sample size) [b]
Santos [27]	R	Hospital-based	1. Post-stroke depression (20) 2. No post-stroke depression (21)
Schneider [50]	RP	Population-based	No dementia (148)
Del Ser [43]	RP	Hospital-based	1. AD (35) 2. AD + CVD (22)
Sinka [18]	RP	Hospital-based	1. Dementia (71) 2. No dementia (22)
Sonnen [9]	RP	Population-based	1. Dementia (75) 2. MCI (47) 3.No dementia (89)
Soontornniyomkij [30]	R	Hospital-based	1. Dementia + severe CAA (18) 2. Dementia + mild CAA (21)
Strozyk [35]	RP	Hospital- and population-based	1. Dementia (131) 2. No dementia (59)
Suter [45]	R	x	1. AD (105) 2. No dementia (79)
Troncoso [24]	RP	Population-based	Elderly +/- dementia (179)
Vinters [36]	RP	Hospital-based	1. +/- dementia/ CVD (20) 2. No dementia, no CVD (3)
Wang [7]	RP	Population-based	1. Age 65-80 (148) 2. Age >80 (102)
White [3]	P	Population-based (selected)	Elderly (285)
White [8]	RP	Population-based	1. Dementia (183) 2. MCI (I70) 3. No dementia (190)
Xuereb [25]	P	Population-based	1. Dementia (47) 2. No dementia (52)
Yip [46]	R	Hospital-based	AD (99)

Studies are listed in alphabetical order. X = unknown/not-specified.

[a] Design: P = prospective, R = retrospective, RP = retrospective from prospective cohort

[b] Diagnosis: VaD = Vascular dementia, NDD = neurodegenerative disease, AD = Alzheimer's disease, MD = Mixed Dementia, DLB = dementia with Lewy bodies, FTD = frontotemporal dementia, CAA = cerebral amyloid angiopathy, PD = Parkinson's Disease, CVD = cerebrovascular disease

Diagnosis based on [c]	Mean age at death (range)	Male (%)
Neuropathology (stroke), DSM (post-stroke depression)	1. 77 (stroke) 2. 78 (stroke)	1. 45 2. 52
Clinical	88	43
Neuropathology	1. 77 2. 83	1. 46 2. 14
Clinical (DSM)	1. 95 2. 95	1. 36 2. 32
Clinical (CASI, DSM)	1. 89 2. 88 3. 84	1. 44 2. 49 3. 37
Clinical (dementia), neuropathology (CAA)	1. 82 (62-97) 2. 84 (69-101)	1. 56 2. 52
Clinical (DSM)	84	35
Neuropathology (NIA, CERAD)	1. 78 (54-98) 2. 72 (57-87)	x
Clinical (DSM)	87	68
Clinical, MRI	1. 80 (68-92) 2. 80 (74-87)	1. 65 2. 67
Age	1. 82 2.92	1. 45 2. 39
Age	85 (74-97)	100
Clinical (CASI)	x	100
Clinical (CAMDEX)	x (>75)	1. 35 2. 30
Neuropathology (NIA)	75	96

[c] Diagnosis based on: NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association criteria for Alzheimer's Disease, DSM = Diagnostic and Statistical Manual of Mental Disorders, NINDS-AIREN = National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria for vascular dementia, CDR = Clinical Dementia Rating scale, CERAD = Consortium to Establish a Registry for Alzheimer's Disease, NIA = National Institute on Aging-Reagan Institute, CASI = Cognitive Assessment Screening Instrument, CAMDEX = Cambridge Diagnostic Examination for the Elderly

Table 2. Microinfarcts in specific diagnostic groups

Study	Sample (size) [a]	Frequency (%)
Reference groups		
Arvanitakis [19]	Controls (233)	25.3
Brayne [22]	Controls (100)	43
De Reuck [40]	Controls (10)	10
Ghebremedhin [34]	Controls (53)	5.7
Schneider [50]	Controls (148)	24
Sinka [18]	Controls (22)	18.2
Sonnen [9]	Controls (89)	29
Suter [45]	Controls (79)	2.5
Vinters [36]	Controls (3)	33
Wang [7]	1. age 65-80 (148) 2. age >80 (102)	1. 20.4 2. 24.2
White [8]	Controls (190)	x
Xuereb [25]	Controls (52)	33.3
	Total: 1229	**Weighted average: 24**
MCI		
Sonnen [9]	MCI (47)	56
White [8]	MCI (I70)	x
	Total: 217	
AD		
Brayne [22]	AD (76)	44
De Reuck [29]	AD (45)	15.6
Erkinjuntti [33]	AD (5)	20
Isojima [39]	AD (63)	68
Kövari [5]	AD (43)	27.9
Okamoto [44]	AD (8)	100
Suter [45]	AD (105)	32.4
Yip [46]	AD (99)	x (rare)
	Total: 409	**Weighted average: 43**

	Size	Characteristics [b]
	Microscopic	x
	x	x
	x	x
	<2 mm	x
	Microscopic	Acute: 4, chronic: 1, 2
	<1 mm	x
	Microscopic	1, 3, 5
	300 µm – 2 mm	x
	Microscopic	1, 4
	Microscopic	x
	Microscopic	1, 3, 5
	x	x
	Microscopic	1, 3, 5
	Microscopic	1, 3, 5
	x	x
	x	x
	Microscopic	1, 3, 4
	Microscopic	x
	Microscopic	x
	100-500 µm	1, 4
	300 µm – 2 mm	x
	< 2 mm	1, 2

Table 2. Microinfarcts in specific diagnostic groups (extended)

Study	Sample (size) [a]	Frequency (%)
VaD		
Brayne [22]	VaD (4)	100
Erkinjuntti [33]	VaD (27)	70.4
Esiri [26]	VaD (24)	63
Haglund [42]	VaD (26)	57.7
Okamoto [44]	VaD (6)	16.7
	Total: 87	**Weighted average: 62**
Mixed dementia		
Ballard [20]	VaD +/- NDD (67)	40
Brayne [22]	AD+VaD (25)	75
Lee [20]	AD + CVD (201)	50
Olichney [28]	1. AD - severe CAA (50) 2. AD - mild CAA (198)	1. 22 2. 9
De Reuck [40]	AD - CAA (12)	42
Del Ser [43]	AD (35), AD+CVD (22)	x
	Total: 553	Weighted average: 33

x = unknown, GM = grey matter, WM = white matter

[a] MCI = mild cognitive impairment, AD = Alzheimer's disease, VaD = Vascular dementia, NDD = neurodegenerative disease, CVD = cerebrovascular disease, CAA = cerebral amyloid angiopathy

[b] Grouped as following:

1. gliotic lesions, including non-cystic lesions, microglial nodules, astrocytosis and fibrous and gemistocytic astrocytes
2. cystic lesions (some described with ragged edges)
3. necrotic lesions and pallor, including focal leukoencephalopathy, foci of myelin loss and tissue devitalisation
4. inflammatory reaction, including histiocytosis, activated macrophages and microglial activation
5. neuronal loss or granular cortical atrophy

	Size	Characteristics [b]
	x	x
	Microscopic	1, 3, 4
	Microscopic	GM: 1, 2, 5; WM: 2
	< 5 mm	3, 4
	100-500 μm	1, 4
	x	GM: 1, 2, 5; WM: 2
	x	x
	x	5
	x	x
	x	x
	<0.5 mm	1, 2

Table 3. Microinfarcts in other diagnostic groups

Study	Sample (size) [a]	Frequency (%)
Dementia/ no dementia combined		
Gold [16]	Dementia /no dementia (156)	41.7
Longstreth [6]	Age>65(+/-dementia) (6189)	19.7
Rossi [41]	AD, dementia, MCI, controls (87)	40
Strozyk [35]	Dementia (131), controls (59)	15.8
Troncoso [24]	Dementia/no dementia (179)	21.8
White [3]	Elderly (285)	19.3 (80.7 negligible)
Dementia not specified		
Arvanitakis [19]	Dementia (192)	36.5
Sonnen [9]	Dementia (75)	63
Xuereb [25]	Dementia (47)	40.5
White [8]	Dementia (183)	x
Sinka [18]	Dementia (71)	54.9
DLB		
Isojima [39]	DLB (25)	40
De Reuck [40]	DLB (8)	17.5
Ghebremedhin [34]	DLB (13)	30.8
Vascular brain damage		
Esiri [26]	CVD (19)	26
Santos [27]	1. Post-stroke depression (20) 2. No post-stroke depression (21)	1. LH 35, RH 55 2. LH 24, RH 52
Soontornniyomkij [30]	1. Dementia + severe CAA (18) 2. Dementia + mild CAA (21)	1. 77.8 2. 33.3
Vinters [36]	+/- dementia/subcortical CVD (20)	60

x = unknown, GM = grey matter, WM = white matter

[a] AD = Alzheimer's disease, MCI = mild cognitive impairment, DLB = dementia with Lewy bodies, CVD = cerebrovascular disease, CAA = cerebral amyloid angiopathy

[b] Grouped as following:

1. gliotic lesions, including non-cystic lesions, microglial nodules, astrocytosis and fibrous and gemistocytic astrocytes

	Size	Characteristics [b]
	<1 mm	x
	Microscopic	x (macroscopically 5)
	x	GM: 1, 2, 5; WM: 2
	Microscopic	1, 2, 3
	Microscopic	x
	50-400 µm, cystic larger	1, 3, 5
	Microscopic	x
	Microscopic	1, 3, 5
	x	x
	Microscopic	1, 3, 5
	<1 mm	x
	Microscopic	x
	x	x
	<2 mm	x
	Microscopic	GM: 1, 2, 5; WM: 2
	Microscopic	3, 5
	< 5 mm	4
	Microscopic	1, 4

2. cystic lesions (some described with ragged edges)
3. necrotic lesions and pallor, including focal leukoencephalopathy, foci of myelin loss and tissue devitalisation
4. inflammatory reaction, including histiocytosis, activated macrophages and microglial activation
5. neuronal loss or granular cortical atrophy

elderly, i.e. the ACT Study[7-9] or the Baltimore Longitudinal Study on Aging.[24] Two of these population-based studies involved specific groups of individuals: i.e. only men (HAAS)[3,8] or people from religious orders (Religious Orders Study19) (Table 1).

The majority of patient studies concerned patients with dementia (AD (n=8), VaD (n=5), Lewy Body Dementia (LBD) (n=3), or different types of dementia mixed (n=5)), with or without a non-demented reference group. Six studies reported on a combination of people with and without dementia described as a single group. Two studies assessed patients with stroke,[26,27] one study elderly without describing their cognitive status.[6] Three papers concerned patients with cerebral amyloid angiopathy (CAA).[28-30]

Thirteen studies (41%) diagnosed the patients primarily on neuropathological criteria.[5,6,21,26,28,29,39-46] The most commonly used neuropathological criteria for AD were the Consortium to Establish a Registry for Alzheimer's Disease (CERAD)[31] and the Braak criteria.[32] Fifteen studies (41%) primarily classified the patients on clinical criteria.[3,7-9,16,18,19,22-25,33-36] The Diagnostic and Statistical Manual of Mental Disorders (DSM)37 was used most frequently, followed by the Cognitive Assessment Screening Instrument (CASI).[38] Four studies based their diagnosis on a combination of neuropathology (cerebrovascular disease, AD, stroke, CAA) and clinical examination (dementia, depression)[20,21,27,30] (Table 1).

The number of included patients varied per study between 14 and 6189, with a mean of 332 and a median of 99 patients. Different definitions of age were used. Age at death was most often used, which varied between a mean of 69 and 95 years of age. One paper reported age at onset of dementia,[21] another the age at which stroke occurred[27] (Table 1).

Pathological assessment method

The areas of the brain that were sampled varied across papers. Eleven studies (34%) investigated only one hemisphere,[19,20,22,24,25,29,33,26,39-41] while 13 studies (41%) assessed both hemispheres.[3,5,7,8,16,18,21,23,26,27,30,42,43] The remainder (25%) did not specify which hemispheres were investigated.[6,9,28,34,36,44-46] Twenty papers (69%) specified a sampling protocol that covered the major parts of the brain, including the frontal, temporal, parietal and occipital lobes, deep white and grey matter, brainstem and sometimes also the cerebellum.[3,7-9,20-22,26,30,33-36,39,41,43,46] The remaining studies had a more limited brain coverage, i.e. only the four lobes[44] or the temporal, parietal and frontal lobes combined with deep grey matter.[5,16,27,28] One study focused on the watershed areas,[45] another study only on one midtemporal and one midparietal section[42] (See supplementary table 2).

A brain slice thickness for macroscopic examination of 10 mm was most common (14 studies, 44%). One study used a brain slice thickness of 5 mm.[36] Brain slice thickness was not specified in 17 papers (53%). The thickness of microscopic slices was 6 µm in three studies, 7 µm or 8 µm in one study each, 10 µm and 20 µm in four studies each and. It was not specified in 19 studies (59 %) (See supplementary table 2).
Three studies reported to have used immunohistochemistry to visualize astrocytes, microglia and macrophages, i.e. with primary antibodies to glial fibrillary acidic protein (GFAP)[36,44] or to Cluster of Differentiation 68 (CD-68).[30,44]

Histological characteristics of microinfarcts

The description of the appearance of microinfarcts varied between the studies from slit-like, triangular, round, barrel-shaped, stellate, granular to wedge-shaped forms. Frequently used terms were foci with neuronal loss (5 studies), gliosis (10 studies), cystic lesions (8 studies) or foci with pallor (2 studies) (Table 2 and 3, Figure 2).
Six studies described features of an inflammatory response, namely activated macrophages,[23,30,33,42,44,50] microglial activation[42] and histiocytosis.[36] Two papers reported to have used immunohistochemistry to define this inflammatory response. Okamoto et al.[44] used infiltration by both GFAP positive astrocytes and CD-68 positive microglia/microphages as a marker of recent occurrence of microinfarcts. In contrast, Soontorniyomkij et al.[30] considered microscopic foci of CD-68 positive macrophage-infiltrated tissue destruction to be old microinfarcts. A few studies only included temporally remote microinfarcts, which they described as cystic or gliotic lesions and foci of pallor.[3,8,9]

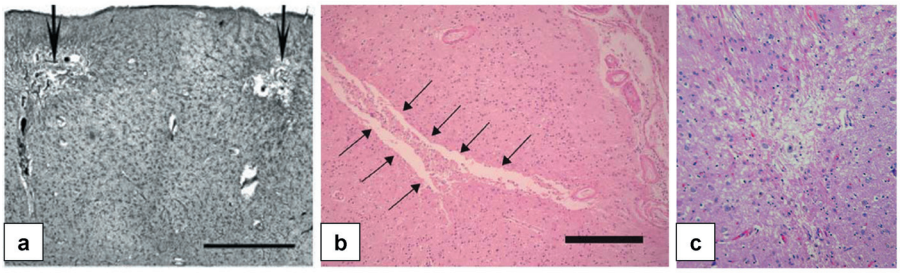


Figure 2. Photomicrographs showing cortical cystic microinfarcts (a, b) and a small focus of collapsed gliotic tissue (c). Material was stained with Globus silver staining (a) and hematoxylin and eosin staining (b, c). Scale bar a: 1000 mm, b: 250 mm. (Images from: Gold et al.[16] (a); Okamoto et al.[44] (b); Strozyk et al.[35] (c))

Four papers only focused on cortical microinfarcts, which some of them described as widespread multiple tiny infarcts.[6,18,20,27]
A substantial number of studies (50%) did not specify the term 'microinfarct', other than naming them 'ischemic lesions'.

Overall, it seems to be that there is no universally accepted histological definition of microinfarcts in neuropathological studies, but that lesions can vary in shape and can appear both cystic or as more gliotic, pale lesions. In the acute stage there can be an inflammatory response.

Size of microinfarcts

Fourteen papers (44%) defined the size of microinfarcts as "not visible with the naked eye" or "only visible upon light microscopy".[3,5-7,9,19,23,24,26,27,33,35,36,39] Some studies (31%) explicitly described sizes of microinfarcts: 50 – 400 μm (with cystic microinfarcts being somewhat larger than noncystic microinfarcts);[3] 100 – 500 μm ;[44] 300 μm – 2 mm ;[45] ≤0.5 mm ;[43] <1 mm;16,18 ≤ 2 mm;[34,46] ≤5 mm.[30,42] The remaining studies (25%) did not define the size of the lesions that they considered microinfarcts[20–22,25,28,29,40,41] (Table 2 and 3).

Distribution of microinfarcts

The compiled data of the included studies indicate that microinfarcts can occur throughout the brain, both in cortical and subcortical grey matter and white matter. Several papers (34%) allowed comparison of lesion occurrence between the cortex and subcortical areas. Some of these studies showed microinfarcts in all cortical and subcortical regions which were assessed,[3,21] or described them more globally in grey and white matter.[7,24,30,36] Only two studies specifically mentioned that there was no difference in appearance of microinfarcts between the cortex and subcortical regions,[21,30] while one study described them to be more common in the subcortical areas.[19] However, most of these studies did not describe if microinfarcts preferentially occurred in cortical or subcortical regions. Regarding specific brain areas, two studies described them to appear most commonly in the parietal and occipital regions.[30,44] Two studies found microinfarcts only in the watershed areas,[35,45] others only in the cortical grey matter.[20,28] White et al.[3] found cortical microinfarcts mostly located toward the base of the grey matter, while Okamoto et al.[44] described them mostly in the superficial layers of the cortex.

Taken together, these results indicate that microinfarcts can appear in all brain areas. Most studies did not specifically compare lesion occurrence in different brain regions. Some found them to be more abundant in the watershed areas or cerebral cortex.

Frequency

Frequencies of cerebral microinfarcts are described in table 2 and 3. Twelve studies included non-demented people, totaling 1229 individuals.[7-9,18,19,22,23,25,34,36,40,45] The frequency of microinfarcts ranged from 3 to 43% (weighted average 24%).

Eight studies concerned patients with AD,[5,22,33,39,40,44-46] with a total sample size of 409 patients (range 5-105). Microinfarcts were detected in 20-100% of the patients, with a weighted average of 43%. One study on patients with AD only described microinfarcts to be 'rare'.[46]

In five studies on vascular dementia, with a total sample size of 87 (range 4-27), the frequency of microinfarcts ranged from 16.7 to 100%, with a weighted average of 62%.[22,26,33,42,44]

Six articles investigated pooled populations of patients with AD and VaD or cerebrovascular disease. In five of these papers, the frequency could be extracted. The summed sample size was 553, and the weighted frequency was 33%.[20-22,28,29,43]

One study reported that microinfarcts were detected in 27 (56%) of 47 patients with mild cognitive impairment (MCI).[9] The frequency of microinfarcts was relatively high in patients with MCI compared to the other diagnostic groups, but this estimate was only based on a small number of patients.

Three studies examined patient groups with vascular brain disease. Esiri et al.[26] identified microinfarcts in 26% of the cases with neuropathological determined cerebrovascular disease, Soontorniyomkij et al.[30] in 78% of the demented patients with severe and in 33% of the patients with mild or absent amyloid angiopathy. Finally, Santos et al.[27] identified similar prevalences in patients with and without post-stroke depression (left hemisphere 35-24% and right hemisphere 55-52%).

The frequency in mixed populations of patients with and without dementia ranged from 16-42% (weighted average 20%).[3,6,16,24,35,41]

One study observed that the mean age was significantly higher in those with microinfarcts.[6]

Overall, the mean age at death of the different study populations was quite homogeneous. Therefore, based on our survey, no definite conclusions can be drawn on the relation between age and microinfarct occurrence. The extensiveness of pathological assessment (i.e. the amount of examined brain regions and slice thickness) seemed to have no influence on the frequency, though it was difficult to compare the studies and frequencies because of the variety of assessment methods and study populations.

Overall, the frequency of microinfarcts varied across studies, but microinfarcts are particularly common in patients with other types of cerebrovascular brain damage and/or dementia.

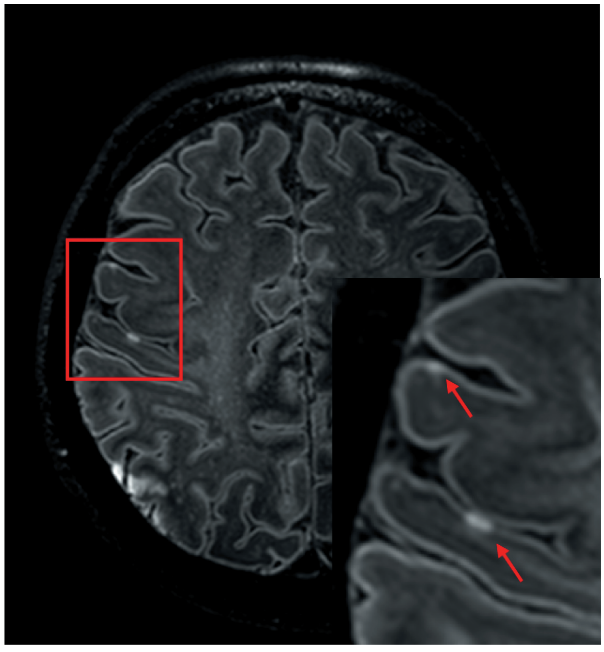


Figure 3 Example of cortical ischemic lesions on MR images obtained at 7Tesla. FLAIR images from a 57 year old woman with a history of atrial fibrillation who presented with aphasia and left-sided hemiparesis based on cortical ischemia in the right middle cerebral artery territory. Note the small cortical hyperintensities (arrows), which represent minute acute ischemic lesions.

Number of microinfarcts per patient

The majority of studies (72%) did not specify lesion numbers per individual patient. Haglund et al.[42] mentioned a maximum of fifteen microinfarcts detected in one patient (only one midtemporal and one midparietal coronal section were examined). Arvanitakis et al.[19] reported a mean number of microinfarcts of 1.6 ± 0.9, 62% of the 129 persons had a single microinfarct and 38% had multiple microinfarcts.

Some studies used a semiquantitative scale, for example 0, 1-2 and >2 microinfarcts (with corresponding prevalence, that ranged from 6% with multiple microinfarcts in non-demented individuals to 31% in patients with dementia);[9] <3; 3; ≥4 in the basal ganglia and thalamus and <3; 3-4; ≥5 in the neocortex (81% of the patients had <3 microinfarcts in both categories, 1% the highest category);[3] or 'multiple'.[20,36] The remaining studies mentioned the number of microinfarcts per patient more precisely: 0-4 in patients with different types of dementia;[33] a mean of 6 microinfarcts (range 1-37) in 14 patients with severe CAA and 3 (range 1-6) in 7 patients with mild CAA;[30] 1-4 per watershed zone area.[45]

In summary, in studies that mentioned the number of microinfarcts per patient, the majority of patients had multiple infarcts. Importantly, it should be noted that it is impossible to examine the whole brain neuropathologically and quite a number of studies investigated only limited sampling sites. Therefore, the actual number of microinfarcts per individual could be up to several hundredfold higher than has been described in these studies.

Clinical correlates of microinfarcts

Because papers examined different study populations, microinfarcts were reported to be associated with various conditions. Several studies reported microinfarcts to be associated with a clinical diagnosis of "dementia",[9,16,18,19,24] neuropathologically confirmed AD,[39,44,45] a clinical diagnosis of VaD[22,26] or "cognitive dysfunction".[3,5,8] However, others found no relationship between microinfarcts and dementia,[22,35] AD pathology[25] or pathologically determined VaD.[44] There were no differences in sample size or pathological assessment method between papers that have or have not found an association with different forms of dementia. Overall, the group of investigated patients with VaD was small. The occurrence of cavitated microinfarcts in the basal ganglia in patients with AD may be modulated by APOE4 genotype.[46]

A number of studies found an association between microinfarcts and other cerebrovascular disease: lacunar infarcts,[36,41] small (<15 ml) macroscopic infarcts,[21] macroinfarcts[6,19,23,24] and leukoencephalopathy.[6,41] There may be an association between microinfarcts and CAA, but reported frequencies are not consistent.[28-30,42]

Finally, a few studies described a relationship between microinfarcts and vascular risk factors (systolic blood pressure, diabetes mellitus, myocardial ischemia).[6,7,47]

DISCUSSION

This systematic review includes 32 papers concerning neuropathological studies on cerebral microinfarcts in patients with cognitive dysfunction or cerebrovascular disease. Microinfarcts are described as gliotic or cystic lesions, sometimes with neuronal loss, and may occur in all brain regions, although the cerebral cortex may be a preferential location. Microinfarcts are commonly described in the elderly, particularly in patients with VaD, AD and cerebrovascular disease. In the majority of cases multiple microinfarcts are observed.

The studies described in this review differed in several aspects. Study populations did not only consist of groups of patients with different types of dementia, but also involved demented and non-demented people together and groups with

different types of cerebrovascular disease. The frequency of microinfarcts seems to increase in patients with dementia (VaD as well as AD) and dementia combined with cerebrovascular disease compared to controls. However, because pathology studies cannot achieve full brain coverage, these numbers are unlikely to be precise estimates of the true prevalence of microinfarcts. For the same reason, it is difficult to draw conclusions on the number of microinfarcts per patient. In the papers that did report microinfarct numbers in their samples, the majority of patients had multiple microinfarcts. Therefore the actual number for the whole brain could be up to hundreds or thousands. About half of the studies used a population-based cohort, the others a hospital-based cohort. In both types of cohorts, sampling bias is inevitable. A limitation of population-based studies is selection bias, due to a limited number of participants. In hospital-based studies, especially from tertiary reference centres, atypical types of dementia could be overrepresented. However, we have not found systematic differences in frequency of microinfarcts between these two types of cohorts.

Finally, we used a broad array of search terms to find as many neuropathological studies on cerebral microinfarcts as possible, and did not use inclusion/exclusion criteria with respect to specific features or definitions of the microinfarcts. This is reflected in the large variability in the characteristics of microinfarcts reported in the different studies, such as size and appearance, but may also be a source of differences in microinfarct frequencies and numbers between studies. As such, this review demonstrates that there is an obvious need for standardisation of neuropathological criteria for microinfarcts, in order to achieve better correspondence of findings from different laboratories in future studies.

The presence of microinfarcts was often correlated to small vessel disease, represented by lacunar infarcts[36,41] and leukoencephalopathy on both neuropathological examination and brain CT or MRI prior to death.[6,41] Furthermore, a few studies assessed patients with CAA and found an association with the occurrence of microinfarcts.[28-30] Others found cortical vessels in immediate proximity to microinfarcts often to be engorged with amyloid.[42] Small vessel disease, as well as amyloid angiopathy, can cause marginal local brain perfusion.[2] Moreover, cortical microinfarcts may be more common in the watershed areas.[35,45]

Microinfarcts and other vascular damage of the cerebral parenchyma with which they are associated, can result in lower cognitive performance. Indeed, microinfarcts seem to be associated with cognitive dysfunction and dementia. An explanation could be that microinfarcts themselves, because of neuronal loss, lead to intellectual decline. Disturbances in neuronal connectivity throughout the brain could also be

the cause of cognitive dysfunction.

Because microinfarcts are correlated to vascular brain damage and dementia, detection of these lesions *in vivo* may have a high potential for future pathophysiological studies in VCI. Detection in vivo would make it possible to investigate their evolution over time and their relationship with the development of cognitive impairment and dementia. Based on the neuropathological findings on microinfarcts, i.e. their widespread appearance, differences in shape and the fact that they can appear as both gliotic and cystic lesions, it will be a challenge to recognize them on MR imaging. Nevertheless, with ultra-high field (7 Tesla) MRI, we have already been able to visualize cortical hyperintense lesions which may represent small ischemic lesions (figure 2). To visualize microinfarcts on brain MRI, one should focus on all brain regions, probably with particular attention for the watershed areas and cerebral cortex. Cystic microinfarcts would be hypointense on FLAIR images and hyperintense on T_2-weighted images. The more gliotic lesions will result in a hyperintense signal on both T_2-weighted and FLAIR images. With the current resolution that can be achieved by a 7 Tesla MRI scanner, microinfarcts are expected to give focal signal intensity changes in only one or a few voxels. Therefore, typical characteristics that can be seen in a histological biopsy will be difficult to recognise. Furthermore, up to the present, an inflammatory response can only be visualised on brain MRI in experimental studies.[48,49] Future research should focus on radiological-pathological correlates in human as well.

Supplementary table 1. Search strings

Pubmed search string

('microinfarct'[tiab] OR 'microinfarction'[tiab] OR 'microinfarcted'[tiab] OR 'microinfarctions'[tiab] OR 'microinfarcts'[tiab] OR 'microischemia'[tiab] OR 'microischemic'[tiab] OR 'micro infarct'[tiab] OR 'micro infarcted'[tiab] OR 'micro infarction'[tiab] OR 'micro infarctions'[tiab] OR 'micro infarcts'[tiab] OR 'micro ischaemia'[tiab] OR 'micro ischaemic'[tiab] OR 'microvascular infarcts'[tiab] OR 'microvascular infarction'[tiab] OR 'microvascular ischemia'[tiab] OR 'microvascular ischaemia'[tiab])

Embase search string

('microinfarct':ti,ab OR 'microinfarction':ti,ab OR 'microinfarcted':ti,ab OR 'microinfarctions':ti,ab OR 'microinfarcts':ti,ab OR 'micro infarct':ti,ab OR 'micro infarcted':ti,ab OR 'micro infarction':ti,ab OR 'micro infarctions':ti,ab OR 'micro infarcts':ti,ab OR ' micro ischaemia':ti,ab OR 'micro ischaemic':ti,ab OR 'microischemia':ti,ab OR 'microischemic':ti,ab OR 'microvascular infarcts':ti,ab OR 'microvascular infarction':ti,ab OR 'microvascular ischemia':ti,ab OR 'microvascular ischaemia':ti,ab)

Supplementary table 2. Pathological assessment method of included studies

Study	Sampling site [a]	Slice thickness	
		Brain slices (mm)	Microscopic slices (μm)
Arvanitakis [19]	F(c), T(c), P(c), hippocampus, cingulate cortex, BG, midbrain	10	6
Ballard [20]	F, T, P, O, DWM, BG, brainstem, hippocampus	x	x
Brayne [22]	F, T, P, O, BG, hippocampus, 'other'	x	10
De Reuck [40]	F, T, BG, brainstem, cerebellum	x	x
De Reuck [29]	F, T, BG, brainstem, cerebellum	x	x
Erkinjuntti [33]	F, P, T, O, DGM, brainstem, cerebellum	10	10
Esiri [26]	F, P, T, DWM, DGM	10	x
Ghebremedhin [34]	F, P, T, O, hippocampus, cingulate cortex, BG, DWM, brainstem, cerebellum	x	10
Gold [16]	T(c), F(c), P(c), hippocampus	10	20
Haglund [42]	Mid T, mid P coronal	x	x
Isojima [39]	each area	10	7
Kövari [5]	F, P, T, hippocampus	10	20
Lee [20]	F, P, T, cingulate cortex, DGM, brainstem, cerebellum	10	x
Longstreth [6]	x	x	x
Okamoto [44]	T(c), F(c), P(c), O(c)	x	x
Olichney [28]	T, F, P, hippocampus, cingulate cortex, midbrain	x	x
Rossi [41]	T, F, P, O, DWM, DGM, brainstem	x	x
Santos [27]	T, F, P, hippocampus	10	20
Schneider [50]	F(c), T(c), P(c), hippocampus, DGM, midbrain	10	6
Del Ser [43]	F(c), P(c), T(c), O(c), hippocampus, WM	10	x
Sinka [18]	T(c), F(c), P(c), O(c), hippocampus	10	20
Sonnen [9]	GM, WM	x	x

Study	Sampling site [a]	Slice thickness	
		Brain slices (mm)	Microscopic slices (μm)
Soontornniyomkij [30]	F, P, T, O, hippocampus, cingulate cortex, cerebellum	x	6
Strozyk [35]	F, P, T, motor cortex, cingulate cortex, calcarine cortex, DGM, brainstem, cerebellum	x	x
Suter [45]	F and P watershed areas	x	x
Troncoso [24]	F, P, T, O, cingulate cortex, DGM, brainstem, cerebellum	x	x
Vinters [36]	All grossly identifiable lesions, cortex, DWM, DGM	5	x
Wang [7]	F, P, T, O, capsula interna, BG, thalamus	x	x
White [3]	38 brain regions	10	8
White [8]	36 brain regions	10	x
Xuereb [25]	F, P, T, O, DGM, brainstem, cerebellum	x	x
Yip [46]	P, T, calcarine cortex, WM, hippocampus, DGM, brainstem	x	10

Studies are listed in alphabetical order. X = unknown/not-specified.

[a] F(c) = frontal (cortical), T = temporal, P = parietal, O = occipital, BG = basal ganglia, (D)WM = (deep) white matter, (D)GM = (deep) grey matter

REFERENCES

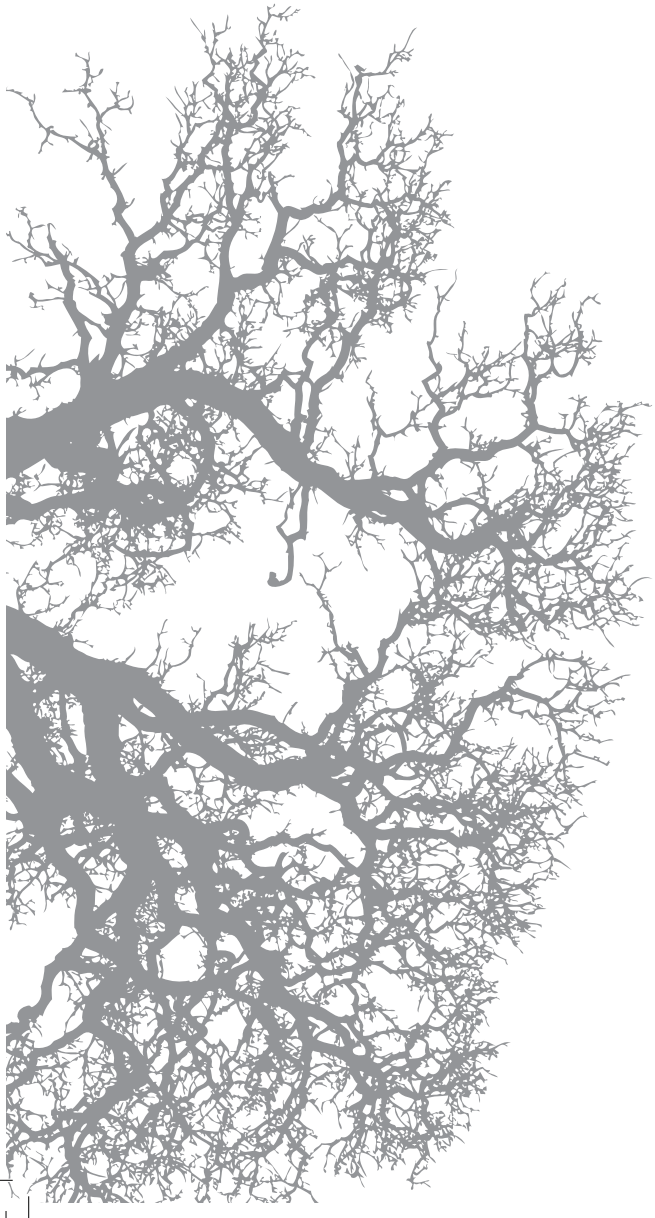
1. O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, Bowler JV, Ballard C, DeCarli C, Gorelick PB, Rockwood K, Burns A, Gauthier S, DeKosky ST. Vascular cognitive impairment. *Lancet Neurol* 2003;2:89-98.
2. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* 2010;9:689-701.
3. White L, Petrovitch H, Hardman J, Nelson J, Davis DG, Ross GW, Masaki K, Launer L, Markesbery WR. Cerebrovascular pathology and dementia in autopsied Honolulu-Asia Aging Study participants. *Ann N Y Acad Sci* 2002;977:9-23.
4. Kalaria RN, Kenny RA, Ballard CG, Perry R, Ince P, Polvikoski T. Towards defining the neuropathological substrates of vascular dementia. *J Neurol Sci* 2004 15;226:75-80.
5. Kövari E, Gold G, Herrmann FR, Canuto A, Hof PR, Bouras C, Giannakopoulos P. Cortical microinfarcts and demyelination affect cognition in cases at high risk for dementia. *Neurology* 2007;68:927-931.
6. Longstreth WT, Jr., Sonnen JA, Koepsell TD, Kukull WA, Larson EB, Montine TJ. Associations between microinfarcts and other macroscopic vascular findings on neuropathologic examination in 2 databases. *Alzheimer Dis Assoc Disord* 2009;23:291-294.
7. Wang LY, Larson EB, Sonnen JA, Shofer JB, McCormick W, Bowen JD, Montine TJ, Li G. Blood Pressure and Brain Injury in Older Adults: Findings from a Community-Based Autopsy Study. *J Am Geriatr Soc* 2009; 57:1975-1981.
8. White L. Brain Lesions at Autopsy in Older Japanese-American Men as Related to Cognitive Impairment and Dementia in the Final Years of Life: A Summary Report from the Honolulu-Asia Aging Study. *J Alzheimers Dis* 2009;18:713-725.
9. Sonnen JA, Larson EB, Crane PK, Haneuse S, Li G, Schellenberg GD, Craft S, Leverenz JB, Montine TJ. Pathological correlates of dementia in a longitudinal, population-based sample of aging. *Ann Neurol* 2007;62:406-413.
10. Sonnen JA, Larson EB, Brickell K, Crane PK, Woltjer R, Montine TJ, Craft S. Different Patterns of Cerebral Injury in Dementia With or Without Diabetes. *Arch Neurol* 2009;66:315-322.
11. Sonnen JA, Larson EB, Walker RL, Haneuse S, Crane PK, Gray SL, Breitner JC, Montine TJ. Nonsteroidal anti-inflammatory drugs are associated with increased neuritic plaques. *Neurology* 2010;75:1203-1210.
12. Kövari E, Gold G, Herrmann FR, Canuto A, Hof PR, Michel JP, Bouras C, Giannakopoulos P. Cortical microinfarcts and demyelination significantly affect cognition in brain aging. *Stroke* 2004;35:410-414.
13. Gold G, Bouras C, Canuto A, Bergallo MF, Herrmann FR, Hof PR, Mayor PA, Michel

JP, Giannakopoulos P. Clinicopathological validation study of four sets of clinical criteria for vascular dementia. *Am J Psychiatry* 2002;159:82-87.

14. Gold G, Giannakopoulos P, Montes-Paixao JC, Herrmann FR, Mulligan R, Michel JP, Bouras C. Sensitivity and specificity of newly proposed clinical criteria for possible vascular dementia. *Neurology* 1997;49:690-694.
15. Gold G, Kövari E, Herrmann FR, Canuto A, Hof PR, Michel JP, Bouras C, Giannakopoulos P. Cognitive consequences of thalamic, basal ganglia, and deep white matter lacunes in brain aging and dementia. *Stroke* 2005;36:1184-1188.
16. Gold G, Giannakopoulos P, Herrmann FR, Bouras C, Kövari E. Identification of Alzheimer and vascular lesion thresholds for mixed dementia. *Brain* 2007;130:2830-2836.
17. Santos M, Gold G, Kövari E, Herrmann FR, Hof PR, Bouras C, Giannakopoulos P. Neuropathological analysis of lacunes and microvascular lesions in late-onset depression. *Neuropathol Appl Neurobiol* 2010;36:661-672.
18. Sinka L, Kövari E, Gold G, Hof PR, Herrmann FR, Bouras C, Giannakopoulos P. Small vascular and Alzheimer disease-related pathologic determinants of dementia in the oldest-old. *J Neuropathol Exp Neurol* 2010;69:1247-1255.
19. Arvanitakis Z, Leurgans SE, Barnes LL, Bennett DA, Schneider JA. Microinfarct pathology, dementia, and cognitive systems. *Stroke* 2011;42:722-727.
20. Lee JH, Olichney JM, Hansen LA, Hofstetter CR, Thal LJ. Small concomitant vascular lesions do not influence rates of cognitive decline in patients with Alzheimer disease. *Arch Neurol* 2000;57:1474-1479.
21. Ballard C, McKeith I, O'Brien J, Kalaria R, Jaros E, Ince P, Perry R. Neuropathological substrates of dementia and depression in vascular dementia, with a particular focus on cases with small infarct volumes. *Dement Geriatr Cogn Disord* 2000;11:59-65.
22. Brayne C, Richardson K, Matthews FE, Fleming J, Hunter S, Xuereb JH, Paykel E, Mukaetova-Ladinska EB, Huppert FA, O'Sullivan A, Dening T. Neuropathological correlates of dementia in over-80-year-old brain donors from the population-based Cambridge city over-75s cohort (CC75C) study. *J Alzheimers Dis* 2009;18:645-658.
23. Schneider JA, Boyle PA, Arvanitakis Z, Bienias JL, Bennett DA. Subcortical infarcts, Alzheimer's disease pathology, and memory function in older persons. *Ann Neurol* 2007;62:59-66.
24. Troncoso JC, Zonderman AB, Resnick SM, Crain B, Pletnikova O, O'Brien RJ. Effect of infarcts on dementia in the Baltimore longitudinal study of aging. *Ann Neurol* 2008;64:168-176.
25. Xuereb JH, Brayne C, Dufouil C, Gertz H, Wischik C, Harrington C, Mukaetova-Ladinska E, McGee MA, O'Sullivan A, O'Connor D, Paykel ES, Huppert FA. Neuropathological findings in the very old. Results from the first 101 brains of a population-based longitudinal study of dementing disorders. *Ann N Y Acad Sci* 2000;903:490-496.

26. Esiri MM, Wilcock GK, Morris JH. Neuropathological assessment of the lesions of significance in vascular dementia. *J Neurol Neurosurg Psychiatry* 1997;63:749-753.
27. Santos M, Gold G, Kövari E, Herrmann FR, Bozikas VP, Bouras C, Giannakopoulos P. Differential impact of lacunes and microvascular lesions on poststroke depression. *Stroke* 2009;40:3557-62.
28. Olichney JM, Ellis RJ, Katzman R, Sabbagh MN, Hansen L. Types of cerebrovascular lesions associated with severe cerebral amyloid angiopathy in Alzheimer's disease. *Ann N Y Acad Sci* 1997;826:493-497.
29. De Reuck J, Deramecourt V, Cordonnier C, Leys D, Maurage CA, Pasquier F. The impact of cerebral amyloid angiopathy on the occurrence of cerebrovascular lesions in demented patients with Alzheimer features: a neuropathological study. *Eur J Neurol* 2011;18:913-918.
30. Soontornniyomkij V, Lynch MD, Mermash S, Pomakian J, Badkoobehi H, Clare R, Vinters HV. Cerebral microinfarcts associated with severe cerebral beta-amyloid angiopathy. *Brain Pathol* 2010;20:459-467.
31. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van BG, Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991;41:479-486.
32. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* 1991;82:239-259.
33. Erkinjuntti T, Haltia M, Palo J, Sulkava R, Paetau A. Accuracy of the clinical diagnosis of vascular dementia: a prospective clinical and post-mortem neuropathological study. *J Neurol Neurosurg Psychiatry* 1988;51:1037-1044.
34. Ghebremedhin E, Rosenberger A, Rub U, Vuksic M, Berhe T, Bickeboller H, De Vos RA, Thal DR, Deller T. Inverse relationship between cerebrovascular lesions and severity of lewy body pathology in patients with lewy body diseases. *J Neuropathol Exp Neurol* 2010;69:442-448.
35. Strozyk D, Dickson DW, Lipton RB, Katz M, Derby CA, Lee S, Wang C, Verghese J. Contribution of vascular pathology to the clinical expression of dementia. *Neurobiol Aging* 2008;31:1710-1720.
36. Vinters HV, Ellis WG, Zarow C, Zaias BW, Jagust WJ, Mack WJ, Chui HC. Neuropathologic substrates of ischemic vascular dementia. *J Neuropathol Exp Neurol* 2000;59:931-945.
37. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fourth edition, text revision (DSM-IV-TR). Washington DC: 2000.
38. Teng EL, Hasegawa K, Homma A, Imai Y, Larson E, Graves A, et al. The Cognitive Abilities Screening Instrument (CASI): a practical test for cross-cultural epidemiological studies of dementia. *Int Psychogeriatr* 1994;6:45-58.

39. Isojima D, Togo T, Kosaka K, Fujishiro H, Akatsu H, Katsuse O, Iritani S, Matsumoto T, Hirayasu Y. Vascular complications in dementia with Lewy bodies: a postmortem study. *Neuropathology* 2006;26:293-297.
40. De Reuck J, Deramecourt V, Cordonnier C, Leys D, Pasquier F, Maurage CA. Prevalence of small cerebral bleeds in patients with a neurodegenerative dementia: a neuropathological study. *J Neurol Sci* 2011;300:63-66.
41. Rossi R, Joachim C, Geroldi C, Combrinck M, Esiri MM, Smith AD, Frisoni GB. Association between subcortical vascular disease on CT and neuropathological findings. *Int J Geriatr Psychiatry* 2004;19:690-695.
42. Haglund M, Passant U, Sjobeck M, Ghebremedhin E, Englund E. Cerebral amyloid angiopathy and cortical microinfarcts as putative substrates of vascular dementia. *Int J Geriatr Psychiatry* 2006;21:681-687.
43. Del Ser T, Hachinski V, Merskey H, Munoz DG. Alzheimer's disease with and without cerebral infarcts. *J Neurol Sci* 2005;231:3-11.
44. Okamoto Y, Ihara M, Fujita Y, Ito H, Takahashi R, Tomimoto H. Cortical microinfarcts in Alzheimer's disease and subcortical vascular dementia. *NeuroReport* 2009;20:990-996.
45. Suter OC, Sunthorn T, Kraftsik R, Straubel J, Darekar P, Khalili K, Miklossy J. Cerebral hypoperfusion generates cortical watershed microinfarcts in Alzheimer disease. *Stroke* 2002;33:1986-1992.
46. Yip AG, McKee AC, Green RC, Wells J, Young H, Cupples LA, Farrer LA. APOE, vascular pathology, and the AD brain. *Neurology* 2005;65:259-265.
47. Sonnen JA, Larson EB, Haneuse S, Woltjer R, Li G, Crane PK, Craft S, Montine TJ. Neuropathology in the Adult Changes in Thought Study: A Review. *J Alzheimers Dis* 2009; 18:703-711.
48. Rausch M, Sauter A, Frohlich J, Neubacher U, Radu EW, Rudin M. Dynamic patterns of USPIO enhancement can be observed in macrophages after ischemic brain damage. *Magn Reson Med* 2001;46:1018-1022.
49. Petry KG, Boiziau C, Dousset V, Brochet B. Magnetic resonance imaging of human brain macrophage infiltration. *Neurotherapeutics* 2007;4:434-442.
50. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* 2007;69:2197-2204.



7 CEREBRAL MICROVASCULAR LESIONS ON 7T MRI: RELATION TO AGE AND TO OTHER MARKERS OF SMALL VESSEL DISEASE

M Brundel

MI Geerlings

YD Reijmer

SJ van Veluw

LEM Wisse

PH van der Veen

PR Luijten

LJ Kappelle

GJ Biessels

On behalf of the Utrecht Vascular Cognitive Impairment (VCI) Study Group

ABSTRACT

Background Cerebral small vessel disease (SVD) is a common feature of brain ageing and can be visualized with magnetic resonance imaging (MRI). Microvascular lesions, i.e. microinfarcts and microbleeds, receive increasing attention as MRI markers of SVD. With ultra-high field MRI microinfarcts can now be visualized in vivo, and the detection of microbleeds is also greatly enhanced. The present study investigated the prevalence of microinfarcts and microbleeds at 7T MRI in persons aged 40-80, and their relationship with vascular risk factors and other markers of SVD.

Methods 103 functionally independent subjects (48% male, mean age 66.6±8.4 years) without major neurological conditions were recruited trough their general practitioners and underwent brain MRI at 7T and conventional field strength (1.5 or 3T). The occurrence of cortical microinfarcts and cerebral microbleeds, determined at 7T FLAIR and T1-weighted images and T_2^*-weighted images respectively, were related to age, vascular risk factors and to lacunar infarcts, white matter hyperintensity (WMH) volumes and brain volumes on conventional MRI.

Results 38% of the persons had cortical microinfarcts; 38% had microbleeds. The number of microbleeds was related to age (r=0.245, p=0.017), but the number of microinfarcts was not (r= -0.014, p=0.892). Neither the presence of microinfarcts nor that of microbleeds was related to vascular risk factors or lacunar infarcts, WMH volumes or total brain volume.

Conclusions This first study on microvascular brain lesions at ultra-high field MRI in persons without major neurological conditions, shows that they are common and already present from middle-aged onwards. Microinfarcts in particular are less clearly related to age than conventional markers of SVD.

INTRODUCTION

Cerebral small vessel disease (SVD) is a common feature of brain ageing and a key contributor to cognitive decline and dementia.[1] On magnetic resonance imaging (MRI), SVD can be visualized as recent small subcortical infarcts, white matter hyperintensities (WMH), lacunes, prominent perivascular spaces, microbleeds and brain atrophy.[1] The occurrence and degree of WMH, lacunar infarcts and brain atrophy increases with age[2-4] and has been related to vascular risk factors, such as hypertension.[5] Importantly, these conventional markers of SVD do not capture the full burden of cerebral microvascular damage. Neuropathological studies have identified microinfarcts as another important feature of SVD.[6,7] These cerebral microinfarcts have been related to cognitive dysfunction and dementia before death.[8] However, their relation to age and vascular risk factors is incompletely known, since only post-mortem data are available. Ultra-high resolution 7T MRI now, for the first time, permits visualization of cortical microinfarcts in vivo[9] and also greatly enhances the detection of cerebral microbleeds.[10] This provides the opportunity to characterize these MRI markers of microvascular brain damage in vivo.

The present study investigated the prevalence of microinfarcts and microbleeds in a sample of subjects without major neurological conditions aged 40-80, and assessed the relationship with age, vascular risk factors and other MRI markers of SVD.

METHODS

Subjects were selected from two related cohorts with similar work-up: 1. The second Utrecht Diabetic Encephalopathy Study (UDES2), a study on MRI-correlates of type 2 diabetes mellitus (T2DM) that included both participants with and without diabetes between 65-80 years of age;[11] and 2. the PREDICT-MR study, a prospective cohort MR study of individuals attending their general practitioner, not selected for the presence of specific medical conditions.[12] Both UDES2 and PREDICT-MR recruited functionally independent subjects from general practices in Utrecht and surrounding areas. Participants were eligible for the present 7T study if they met the following criteria: 1) age above 40; 2) no dementia or other known major neurological conditions; 3) no recent non-disabling stroke (<2 years) or any disabling stroke; 4) no contra-indications for 7T MRI; 5) the 7T MRI scan included a fluid attenuated inversion recovery (FLAIR) and T1-weighted sequence, or a T2*-weighted sequence, needed for detection of microvascular lesions.

The UDES2 included 61 participants without DM. In addition, a random sample of patients with DM (n=9) were selected for the present study to ensure that the prevalence of DM was in accordance with the prevalence of DM in the Dutch population (14-21%).[13] Of these 70 participants, 13 had no (complete) 7T MRI scan (1 with and 12 without T2DM). Therefore, 57 participants were included.

From the 50 participants of the PREDICT-MR study with a 7T MRI, 46 had a FLAIR and T1-weighted sequence, or a T_2^*-weighted sequence and met our inclusion criteria. Hence, the total study sample consisted of 103 subjects (see Figure 1).

On the day of the 7T scan all subjects underwent a standardized evaluation, including medical history, physical and neurological examination and laboratory testing. On the same day, they also underwent regular 3T (UDES2) or 1.5T (PREDICT-MR) MRI. The 7T MRI was used for the detection of microvascular lesions. The 1.5/3T MRIs were used for the detection of WMHs, brain infarcts and the determination of brain volumes and intracranial volumes.

Both studies were approved by the medical ethics committee of the University Medical Center Utrecht and all subjects gave written informed consent.

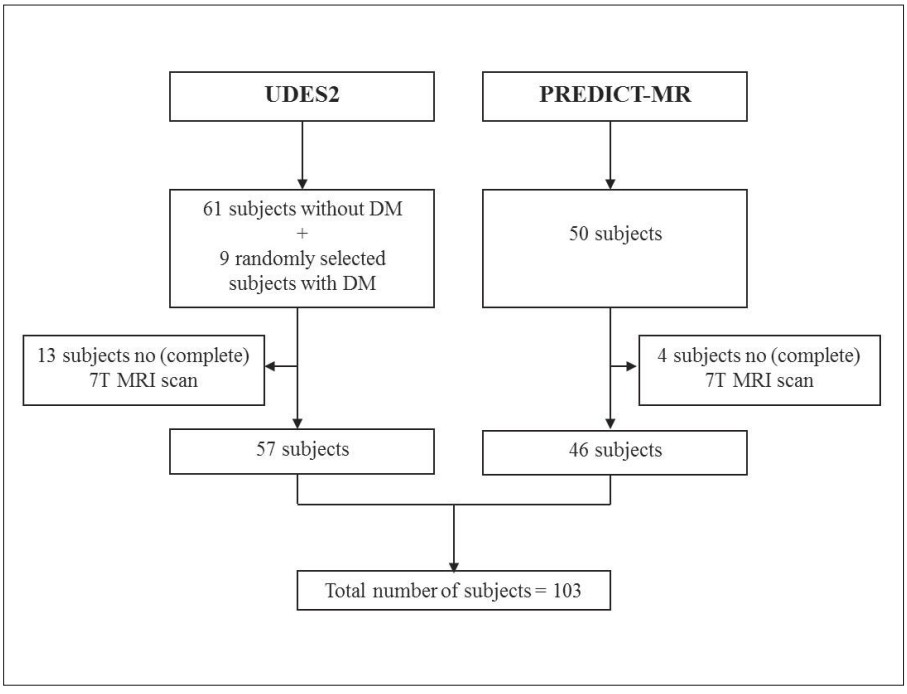


 Figure 1. Flow chart of study participants

MRI scanning protocol

Scans for both cohorts were acquired on a 7T MR system (Philips Healthcare, Cleveland, OH, USA) with a volume transmit and 16 or 32-channel receive head coil (Nova Medical, Wilmington, MA, USA), with a standardized protocol including: 1. dual-echo T2*-weighted sequence (repetition time (TR)/first echo time (TE)/ second TE = 20/6.9/15.8 ms, reconstructed voxel size 0.39 x 0.39 x 0.35 mm^3; 2. volumetric (3D) T1-weighted sequence (TR/TE 4.8/2.2 ms, reconstructed voxel size 0.66 x 0.66 x 0.50 mm^3);[3]. 3D FLAIR (TR/inversion time (TI)/TE: 8000/2325/300 ms, reconstructed voxel size 0.49 x 0.49 x 0.40 mm^3).

In the UDES2 the 3T MRI (Philips Medical Systems, Best, the Netherlands) protocol included, among others, a FLAIR sequence (TR/TI/TE 11000/2800/125 ms, reconstructed voxel size 0.96 x 0.95 x 3.00 mm^3), a 3D T1-weighted sequence (TR/TE 7.9/4.5 ms, voxel size 1.00 mm3 isotropic) and a dual-echo T2-weighted sequence (TR/TE 3198/19;140 ms, reconstructed voxel size 0.96 x 0.95 x 3.00 mm^3).

In the PREDICT-MR the 1.5T MRI (Philips Medical Systems, Best, the Netherlands) protocol included, among others, a FLAIR sequence (TR/TI/TE 4800/1600/317 ms, reconstructed voxel size 0.98 x 0.98 x 0.55 mm^3), a 3D T1-weighted sequence (TR/TE 6.8/3.1 ms, reconstructed voxel size 0.98 x 0.98 x 0.55 mm^3) and a dual-echo T2-weighted sequence (TR/TE 2200/11;100 ms, reconstructed voxel size 0.90 x 0.90 x 4.00 mm^3).

Detection of microvascular lesions

Microvascular lesions were rated visually on 7T MRI scans by two independent raters, blinded to clinical information. In case of disagreement, consensus was obtained in a consensus meeting.

Cortical microinfarcts were defined as either small hyperintense (probably gliotic) lesions or hypointense lesions with a hyperintense rim (probably cystic) on the FLAIR image, corresponding with a hypointense lesion on the T1-weighted sequence (as previously described,[8] but without use of a T2-weighted sequence, because that was not available for all subjects). Each lesion had to be detectable on at least two views (sagittal, coronal and transversal), had to be ≤3mm in size, and restricted to the cortex to be classified as a microinfarct. Due to low signal-to-noise ratio on the 7T FLAIR images in the temporal lobes and cerebellum, these areas were not evaluated. Two raters marked all possible and probable microinfarcts. In a consensus meeting it was decided which lesions eventually were classified as microinfarcts.

Microbleeds were detected on the T2*-weighted images by the previously described semi-automatic method based on the radial symmetry transform (RST).[14]

The method was slightly modified by incorporating minimum intensity projection images. This improves the sensitivity and reduces the number of suspected microbleed locations.[15] The RST result was then censored visually to select true microbleeds.

The inter-rater agreement was excellent for number of microbleeds (ICC (95% CI): 0.88 (0.83; 0.92)) and fair for number of microinfarcts (ICC=0.43 (0.25; 0.58)) The lower ICC for microinfarcts can be explained partially by the fact that all possible microinfarcts were also marked by each rater.

Other MRI measures

WMHs were outlined manually on the 1.5T or 3T FLAIR images and their volumes were calculated. Brain infarcts were rated visually on FLAIR and T1-weighted images and classified as large vessel infarcts (>1.5 cm) or lacunar infarcts.[1] Total brain volumes were determined using the Statistical Parametric Mapping software package (SPM12, www.fil.ion.ucl.ac.uk/spm), executed in Matlab (MathWorks, Natick, MA, USA) and visually checked. Volumes were expressed as a percentage of intracranial volume (ICV), which was outlined manually on the T2-weighted images.

Cardiovascular risk factors

Cardiovascular risk factors were assessed by questionnaires, a standardized interview, physical examination and laboratory testing. Risk factors included in our analyses were systolic and diastolic blood pressure (mean of three measurements), current smoking, diabetes mellitus, serum total cholesterol and a history of a cardiovascular events (defined as a clinical history of myocardial infarction, stroke (not including TIA) or endovascular or surgical treatment of carotid, coronal or peripheral arterial disease).

Statistical analyses

The relationships between number of (microvascular) lesions and WHM volumes and age were measured with Spearman's rank correlation coefficient, because of non-normal distribution of the data. The relationships between total brain volumes and age were examined with linear regression analyses. The relationships between the presence of microinfarcts or microbleeds (as dependent variable) and age, sex, cardiovascular risk factors and other markers of SVD (lacunes, WMH volumes and total brain volume), were assessed with logistic regression analyses, adjusted for age and sex. WMH volume was naturally log transformed because of non-normal distribution.

RESULTS

Table 1 shows the characteristics of all 103 participants. Mean age was 66.6 ± 8.4 years and 48% were male. As expected, considering the different inclusion criteria with respect to age, the two cohorts differed significantly in mean age (UDES2: 71 ± 4 years; PREDICT-MR: 61 ± 9 years), mean systolic blood pressure (UDES2: 147 ± 21 mm Hg; PREDICT-MR: 135 ± 15 mm Hg) and use of cholesterol lowering drugs (UDES2: 44%; PREDICT-MR: 24%) (all p<0.05). Furthermore, the groups differed significantly in sex distribution (UDES2: 58% male; PREDICT-MR: 35% male, p=0.020).

Ninety-seven persons had FLAIR and T1-weighted sequence, required to rate cortical microinfarcts. Cortical microinfarcts were present in 37 persons (38%). Microinfarcts were found in all age categories, varying from 24-50%. The number of microinfarcts in one person ranged from 0-5; most subjects with microinfarcts had a single lesion (27 persons) (table 2). The number of microinfarcts was not related to age (correlation coefficient: -0.014, p=0.892) (figure 2). Logistic regression analyses did not show a relationship between the presence of microinfarcts and blood pressure, cholesterol levels, diabetes mellitus and current smoking, or with other MRI markers of SVD (table 3).

Ninety-four persons had a T2*-weighted MR sequence, required to rate microbleeds. Cerebral microbleeds were present in 36 persons (38%). The prevalence ranged from 30-51% across age categories. Number of microbleeds ranged from 0-6. Most subjects with microbleeds had a single lesion (20 persons) (table 2). Of those with microbleeds, twenty-four subjects (67%) showed strictly lobar microbleeds; 4 (11%) showed strictly infratentorial/deep microbleeds. There was a significant correlation between number of microbleeds and age (correlation coefficient: 0.245, p=0.017) (figure 2). The presence of microbleeds was not related to vascular risk factors or other MRI markers of SVD either (table 4). There was no correlation between number of microbleeds and number of microinfarcts (correlation coefficient: -0.043, p=0.688).

Regarding conventional markers of small vessel disease, the presence of lacunar infarcts at 1.5T / 3T MR field strength increased clearly with age (correlation coefficient for number of lacunes: 0.297, p=0.002), as expected. Large vessel infarcts were relatively uncommon (3 persons). WMH and total brain volumes were also related to age (correlation coefficient for WMH: 0.558; unadjusted regression coefficient B for total brain volume (95% CI): -0.45 (-0.57; -0.32), both p<0.01) (figure 2).

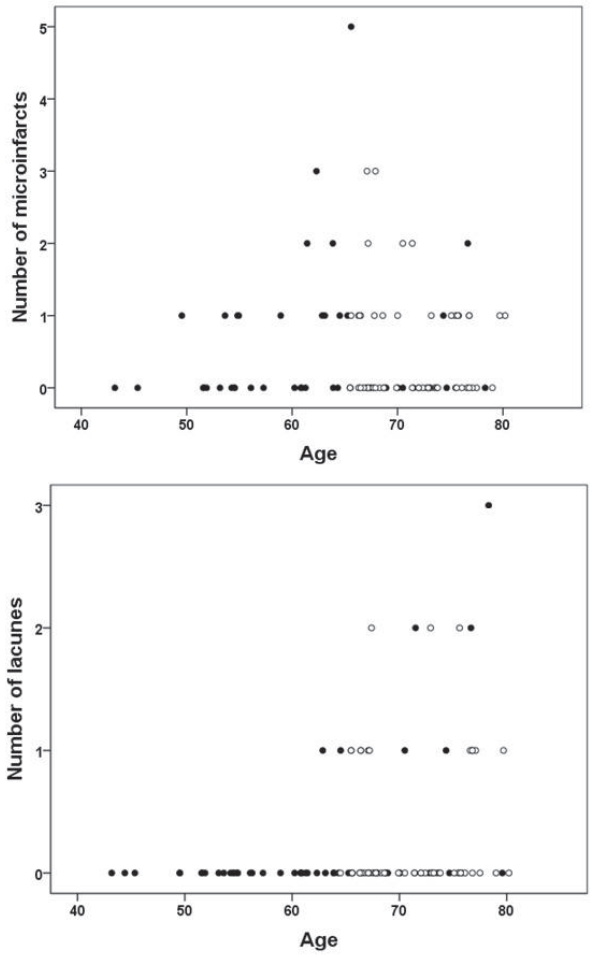
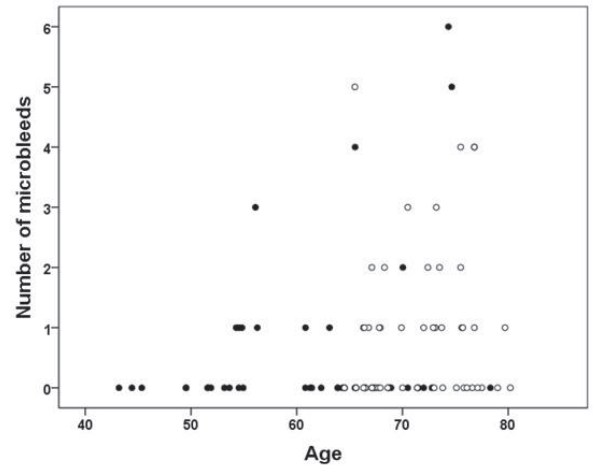
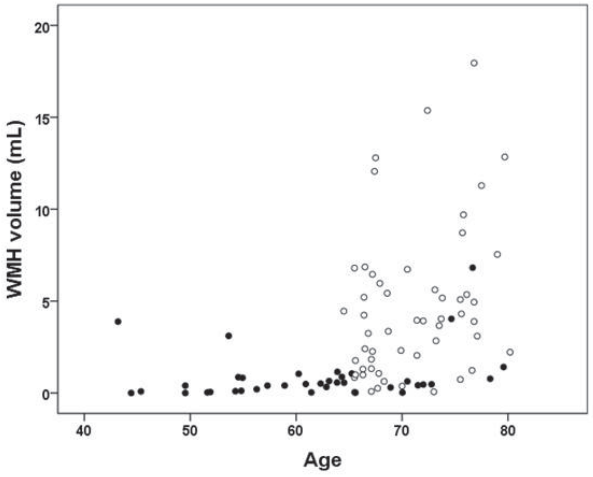


Figure 2. Number of microinfarcts, microbleeds and lacunes and white matter hyperintensity (WMH) volumes and total brain volumes (TBV; expressed as percentage of intracranial volume (ICV)) related to age. Data are expressed for participants of the UDES (○) and the PREDICT-MR study (●). The number of microinfarcts was not related to age (correlation coefficient: -0.014, p=0.892) The other MRI markers were significantly related to age: number of microbleeds (correlation coefficient: 0.245, p=0.017), the number of lacunar infarcts (correlation coefficient: 0.297, p=0.002), WMH volumes (correlation coefficient 0.558; p=0.000) and TBV (regression coefficient B (95% CI): -0.45 (-0.57; -0.32), p<0.01).


Number of microbleeds
0
1
2
3
4
5
6
40
50
60
70
80
Age


WMH volume (mL)
0
5
10
15
20
40
50
60
70
80
Age

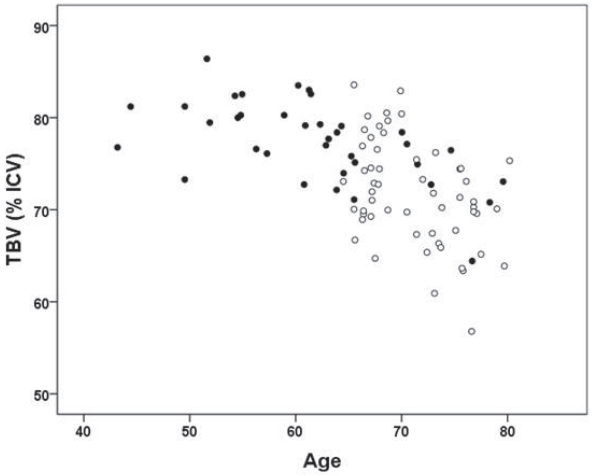

TBV (% ICV)
50
60
70
80
90
40
50
60
70
80
Age

Table 1 Subject characteristics

	Subjects (n=103)
Age (years)	66.6 ± 8.4
Sex (% male)	49 (48)
Systolic blood pressure (mm Hg)	142 ± 19
Diastolic blood pressure (mm Hg)	81 ± 9
Antihypertensive medication	53 (52)
Total cholesterol (mmol/L)	5.5 ± 1.0
Cholesterol lowering drugs	36 (35)
Current smoking	13 (13)
BMI (kg/m2)	26.5 ± 3.8
Antithrombotic medication	28 (27)
Fasting glucose (mmol/L)	5.8 ± 1.0
Diabetes mellitus	12 (12)
Cardiovascular event [a]	7 (7)

Data are presented as mean ± SD or n (%)

[a] A history of a cardiovascular event was defined as a clinical history of myocardial infarction, stroke (not including TIA) or endovascular or surgical treatment of carotid, coronal or peripheral arterial disease

Table 2 MRI characteristics in different age categories

	40-50 y n=5	50-60 y n=14	60-70 y n=45	70-80 y n=39
Microvascular lesions (7T MRI)				
Cortical microinfarcts				
No. of MR scans rated	3	13	42	39
Presence	1 (33)	4 (31)	18 (43)	14 (36)
No.	0 (0-1)	0 (0-1)	0 (0-5)	0 (0-2)
Microbleeds				
No. of MR scans rated	5	12	40	37
Presence	0 (0)	5 (42)	12 (30)	19 (51)
No.	0 (0-0)	0 (0-3)	0 (0-5)	1 (0-6)
Other MRI markers (1.5/3T MRI)				
Lacunar infarcts (n=103)	0 (0)	0 (0)	9 (20)	12 (32)
Large vessel infarcts (n=103)	0 (0)	1 (7)	2 (4)	0 (0)
WMH volumes, mL (median (IQR)) (n=94)	0.09 (0.01;2.14)	0.30 (0.09; 0.84)	1.15 (0.56; 4.35)	4.03 (0.95; 7.18)
Total brain volume (%ICV) (n=90)	78.1 ± 3.8	80.4 ± 3.1	75.5 ± 5.3	70.0 ± 5.3

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

WMH = white matter hyperintensity; ICV = intracranial volume

Table 3 Relationship between microinfarcts and vascular risk factors and other MRI characteristics

	No microinfarcts [a] n=60
Age (years)	66.9 ± 8.5
Sex (male)	24 (40)
Vascular risk factors	
Systolic blood pressure, mm Hg (% Tx)	143 ± 19 (57)
Diastolic blood pressure, mm Hg (% Tx)	81 ± 9 (57)
Cholesterol mmol/L (% Tx)	5.4 ± 1.0 (38)
Diabetes mellitus	7 (12)
Current smoking	7 (12)
MRI markers	
Microbleeds (%; range)	40; 0-5
Lacunar infarcts (%, range)	20; 0-3
WMH volumes (mL) (median, IQR) [c]	1.31 (0.47; 5.00)
Total brain volume (%ICV)	74.0 ± 6.3

IQR = interquartile range; ICV = intracranial volume; Tx = treatment, with either antihypertensive drugs or lipid lowering drugs

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

[b] OR (95% CI) for having ≥1 microinfarct, adjusted for age and sex (systolic and diastolic blood pressure per 10 mm Hg increase; BMI per 5 kg/m2; cholesterol, WMH volumes and TBV per SD increase)

[c] WMH volumes were natural log-transformed

	1 microinfarct [a]	>1 microinfarct [a]	Risk of ≥1 microinfarct [b]
	67.7 ± 8.3	67.4 ± 4.6	1.00 (0.95-1.06)
	15 (56)	6 (60)	1.95 (0.83-4.57)
	144 ± 19 (48)	133 ± 19 (50)	0.90 (0.71-1.13)
	82 ± 9 (48)	79 ± 15 (50)	1.01 (0.64-1.59)
	5.7 ± 1.0 (30)	5.6 ± 0.8 (30)	1.27 (0.83-1.95)
	2 (7)	1 (10)	0.77 (0.18-3.30)
	2 (7)	3 (30)	1.00 (0.28-3.60)
	46; 0-6	22; 0-3	0.90 (0.36-2.23)
	30; 0-1	10; 0-2	1.15 (0.41-3.23)
	2.31 (0.63; 5.26)	1.84 (0.27; 6.59)	1.28 (0.75-2.19) [c]
	73.9 ± 5.9	73.3 ± 5.2	0.99 (0.56-1.74)

Table 4 Relationship between microbleeds and vascular risk factors and other MRI characteristics

	No microbleeds [a] n=58
Age (years)	65.3 ± 9.1
Sex (male)	25 (43)
Vascular risk factors	
Systolic blood pressure, mm Hg (% Tx)	141 ± 19 (53)
Diastolic blood pressure, mm Hg (% Tx)	81 ± 9 (53)
Cholesterol mmol/L (% Tx)	5.5 ± 1.0 (35)
Diabetes mellitus	5 (9)
Current smoking	6 (10)
MRI markers	
Microinfarcts (%; range)	38; 0-5
Lacunar infarcts (%; range)	17; 0-3
WMH volumes (mL) (median (IQR)) [c]	1.23 (0.44; 4.81)
Total brain volume (%ICV)	74.2 ± 5.9

IQR = interquartile range; ICV = intracranial volume; Tx = treatment, with either antihypertensive drugs or lipid lowering drugs

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

[b] OR (95% CI) for having ≥1 microbleed, adjusted for age and sex (systolic and diastolic blood pressure per 10 mm Hg increase; BMI per 5 kg/m2; cholesterol, WMH volumes and TBV per SD increase).

[c] WMH volumes were natural log-transformed.

	1 microbleed [a] n=20	>1 microbleed [a] n=16	Risk of ≥1 microbleed [b]
	67.4 ± 7.9	71.0 ± 5.5	1.06 (1.00-1.12)
	10 (50)	10 (63)	1.48 (0.63-3.48)
	146 ± 43 (45)	138 ± 19 (44)	0.94 (0.74-1.19)
	82 ± 9 (45)	78 ± 7 (44)	0.83 (0.50-1.38)
	5.6 ± 1.3 (50)	5.5 ± 0.8 (25)	1.04 (0.69-1.57)
	6 (30)	1 (6)	2.63 (0.74-9.34)
	3 (15)	3 (19)	1.40 (0.40-4.93)
	42; 0-3	31; 0-2	0.90 (0.36-2.24)
	25; 0-2	19; 0-1	1.00 (0.34-3.01)
	3.57 (0.81; 5.31)	3.85 (0.49; 6.75)	1.08 (0.63-1.84) [c]
	73.0 ± 6.7	73.3 ± 5.1	1.14 (0.64-2.03)

DISCUSSION

Ultra-high field MRI in functionally independent older adults without major neurological conditions, shows that microinfarcts and microbleeds are very common and already present in middle-aged individuals. Microinfarcts in particular are less clearly related to age than microbleeds and conventional markers of SVD.

To the best of our knowledge, this is the first in vivo study on microinfarcts in relation to age in non-demented persons. Previous estimates on the occurrence of microinfarcts in the general population are solely based on autopsy studies. A systematic review of these studies reported frequencies of 24% in non-demented older individuals.[16] In addition, important clinical correlates of microinfarcts have been reported, such as lower cognition,[17] dementia,[17] and lacunar infarcts and white matter lesions on CT prior to death.[7] These autopsy studies, however, provide limited data on the relationship between microinfarcts and age or vascular risk factors. We showed that microinfarcts can now be visualized in vivo, and previously validated these MR lesions with histopathological examination of postmortem tissue.[9] In our study, microinfarcts at 7T MRI appear to be very common, even in younger persons without dementia. Notably, they were unrelated to age. In neuropathological studies, it is obviously only possible to investigate a relationship with age at death. Although a relationship between the presence of microinfarcts at autopsy and a higher age at death has been reported, the mean difference in age between persons with and without microinfarcts was only 3 years (with a mean age above 80) in more than 6000 examined brains.[6] Furthermore, we observed no significant associations with other MR markers of SVD, while in autopsy studies an association between microinfarcts and other signs of cerebrovascular disease has been reported.[6,18] The apparent discrepancy between the links between microinfarcts and age and other SVD markers in our study and in these earlier neuropathological studies may be that the latter investigated much older patients (mean age at death mostly above 80), with a high probability of ageing related abnormalities and end-stage disease, including both Alzheimer's pathology and cerebrovascular disease. Along these lines, in recent in vivo 7T MRI studies, one study reported no increased occurrence of microinfarcts in patients with early Alzheimer's disease (AD) compared to controls,[19] while in another study in patients with more advanced AD microinfarcts were more prevalent compared to controls.[20] It seems to be a paradox that on the one hand microinfarcts are frequently seen, also in younger persons without cerebrovascular disease or cognitive disturbances, but on the other hand that they are associated with dementia and cerebrovascular disease. One explanation could

be that at the population level, microinfarcts are not a manifestation of disease, but rather a marker for future risk of stroke or dementia. In other words, they could be a marker of other pathophysiological processes which causally lead to cognitive decline or stroke. Another explanation could be that they lead to dementia through synergistic or additive effects together with other disease processes. Hence, the relation with dementia would only become evident when these other processes also occur. Finally, the relation between microinfarcts and disease could be not-linear, i.e. persons with a mild lesion load will not develop cognitive symptoms, but patients with a higher lesion load do. Longitudinal studies have the possibility to unravel the natural course of microinfarcts and their clinical implications also in earlier stages of disease.

Previous studies on microbleeds are mainly based on regular field strength (1-3 T MRI). Higher field strength significantly improves the detection of cerebral microbleeds and has been found to identify more individuals with microbleeds as well as a higher number of microbleeds in these individuals.[10,21] Indeed, the prevalence we found (38%) is higher than previously reported at regular field strength in the general population.[22,23] Furthermore, the microbleeds we detected with our sensitive imaging method, were related to age, in line with the literature.[23-25] However, in previous studies reported odds ratios were 1.06 (1.05-1.07) per 1 year[23] and 1.39 (1.17-1.66) per 5-year increase of age,[24] i.e. higher than in our study (OR 1.06 (1.00-1.12) per 5-year increase of age). This difference could be the result of the higher sensitivity of our imaging method. Possibly, we detected smaller lesions, also in younger persons. Previous studies at regular field strengths have linked the presence of microbleeds to vascular risk factors, but not invariably. A meta-analysis that included the earliest studies showed that microbleeds appear to be associated with hypertension (OR 3.9 (2.4-6.4)) and diabetes mellitus (OR 2.2 (1.2-4.2)) in the general population.[22] However, in more recent population-based cohorts, no or weak relationships were found, in line with our study. In the Framingham study, for example, no relation between microbleeds and smoking, and weak relations between microbleeds and hypertension and diabetes have been reported;[25] in the Rotterdam Study, only deep and infratentorial microbleeds were associated with hypertension, systolic blood pressure, and smoking.[23] Studies in the general population yielded conflicting results regarding possible relationships with other imaging markers of SVD as well. Some reported an association between microbleeds and lacunes[26,27] or higher WMH scores,[26] but others found only a relationship with deep or infratentorial microbleeds[23] or no relationship at all,[24] in line with our results. Thus, microbleeds are observed in healthy individuals, but are not strongly related to other markers of

SVD in these persons. However, they have been related to cognitive performance in population-based samples.[28,29] On the other hand, they are more common in persons with dementia or stroke, but in these patient groups microbleeds are not clearly related to cognitive performance.[30,31] It appears that at the population-level, similarly to microinfarcts, microbleeds could be a marker of future risk for stroke and cognitive decline. At the same time, in a subgroup, they could be an early manifestation of a pathophysiological process, e.g. amyloid angiopathy in the context of AD. In patients with stroke or dementia, the overall burden of other vascular and neurodegenerative pathologies will be higher, which may mask the relatively subtle relation between microbleeds and cognition.

A major strength of this study is the use of advanced techniques to assess microvascular lesions at 7T MRI. Another strength is the large sample size for a 7T MRI study and the wide age range of the study population. MRI scans have the advantage over neuropathology that the whole brain, except for the temporal lobes, can be evaluated in a single examination. There are also limitations. The scoring of microvascular lesions is rater dependent, and the results of both raters on microinfarcts showed moderate agreement. However, raters were blinded to clinical information and therefore we do not expect systematic errors. Moreover, our MRI protocol can only detect the largest microinfarcts (≥1 mm),[9] while neuropathology detects much smaller lesions (≥50 μm). It still needs to be established if the lesions that are detectable by MRI are a good representation of the complete lesion load in the brain. Finally, potential selection bias may have arisen because of the stringent contra-indications for 7T MRI, although relationships between other MRI markers of SVD and age were in line with the literature, which supports the validity of our observations.

In conclusion, neuropathological studies point to the relevance of microvascular lesions, in particular microinfarcts, in ageing related cognitive decline and dementia. Our study shows that these lesions are common and already present in middle-aged persons, and that their occurrence is independent of other signs of SVD. SVD is an umbrella term for different types of vascular brain damage, and probably the microvascular lesions we detected reflect pathophysiological processes that are different from other markers of SVD.

REFERENCES

1. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, Lindley RI, O'Brien JT, Barkhof F, Benavente OR, Black SE, Brayne C, Breteler M, Chabriat H, Decarli C, de Leeuw FE, Doubal F, Duering M, Fox NC, Greenberg S, Hachinski V, Kilimann I, Mok V, Van Oostenbrugge R, Pantoni L, Speck O, Stephan BC, Teipel S, Viswanathan A, Werring D, Chen C, Smith C, van Buchem M, Norrving B, Gorelick PB, Dichgans M. STandards for ReportIng Vascular changes on nEuroimaging (STRIVE v1). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* 2013;12:822-838.
2. De Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J Neurol Neurosurg Psychiatry* 2001;70:9-14.
3. Scahill RI, Frost C, Jenkins R, Whitwell JL, Rossor MN, Fox NC. A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Arch Neurol* 2003;60:989-994.
4. Bejot Y, Catteau A, Caillier M, Rouaud O, Durier J, Marie C, Di Carlo A, Osseby GV, Moreau T, Giroud M. Trends in incidence, risk factors, and survival in symptomatic lacunar stroke in Dijon, France, from 1989 to 2006: a population-based study. *Stroke* 2008;39:1945-1951.
5. Grau AJ, Weimar C, Buggle F, Heinrich A, Goertler M, Neumaier S, Glahn J, Brandt T, Hacke W, Diener HC. Risk factors, outcome, and treatment in subtypes of ischemic stroke: the German stroke data bank. *Stroke* 2001;32:2559-2566.
6. Longstreth WT, Jr., Sonnen JA, Koepsell TD, Kukull WA, Larson EB, Montine TJ. Associations between microinfarcts and other macroscopic vascular findings on neuropathologic examination in 2 databases. *Alzheimer Dis Assoc Disord* 2009;23:291-294.
7. Rossi R, Joachim C, Geroldi C, Combrinck M, Esiri MM, Smith AD, Frisoni GB. Association between subcortical vascular disease on CT and neuropathological findings. *Int J Geriatr Psychiatry* 2004;19:690-695.
8. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisble lesions. *Lancet Neurol* 2012;11:272-282.
9. Van Veluw SJ, Zwanenburg JJM, Engelen-Lee JY, Spliet WGM, Hendrikse J, Luijten PR, Biessels GJ. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. *J Cereb Blood Flow Metab* 2013;33:322–329.
10. Conijn MM, Geerlings MI, Biessels GJ, Takahara T, Witkamp TD, Zwanenburg JJ, Luijten PR, Hendrikse J. Cerebral microbleeds on MR imaging: comparison between

1.5 and 7T. *AJNR Am J Neuroradiol* 2011;32:1043-1049.

11. Reijmer YD, Brundel M, de Bresser J, Kappelle LJ, Leemans A, Biessels GJ; Utrecht Vascular Cognitive Impairment Study Group. Microstructural white matter abnormalities and cognitive functioning in type 2 diabetes: a diffusion tensor imaging study. *Diabetes Care*. 2013;36:137-144.
12. Wisse LE, Gerritsen L, Zwanenburg JJ, Kuijf HJ, Luijten PR, Biessels GJ, Geerlings MI. Subfields of the hippocampal formation at 7 T MRI: in vivo volumetric assessment. *Neuroimage*. 2012;61:1043-1049
13. National Compass of Public Health, 2011. Diabetes Mellitus: incidence, prevalence and mortality according to age and sex. Available from: Nationaalkompas.nl. Accessed in 2013.
14. Kuijf HJ, de Bresser J, Geerlings MI, Conijn MM, Viergever MA, Biessels GJ, Vincken KL. Efficient detection of cerebral microbleeds on 7.0 T MR images using the radial symmetry transform. *Neuroimage* 2012;59:2266-2273.
15. Kuijf HJ, Brundel M, de Bresser J, van Veluw SJ, Heringa SM, Viergever MA, Biessels GJ, Vincken KL. Semi-Automated Detection of Cerebral Microbleeds on 3.0 T MR Images. *PLoS One*. 2013;8:e66610.
16. Brundel M, de Bresser J, van Dillen JJ, Kappelle LJ, Biessels GJ. Cerebral microinfarcts: a systematic review of neuropathological studies. *J Cereb Blood Flow Metab*. 2012;32:425-436.
17. Arvanitakis Z, Leurgans SE, Barnes LL, Bennett DA, Schneider JA. Microinfarct pathology, dementia, and cognitive systems. *Stroke*. 2011;42:722-727.
18. Vinters HV, Ellis WG, Zarow C, Zaias BW, Jagust WJ, Mack WJ, Chui HC. Neuropathologic substrates of ischemic vascular dementia. *J Neuropathol Exp Neurol* 2000;59:931-945.
19. Van Veluw SJ, Heringa SM, Kuijf HJ, Koek HL, Luijten PR, Biessels GJ. Cerebral cortical microinfarcts at 7Tesla MRI in patients with Early Alzheimer's Disease. *J Alzheimers Dis* 2014;39:163-167.
20. Van Rooden S, Goos JD, 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* 2014;270:205-211.
21. 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 Neurol* 2009;8:165-174.
22. Cordonnier C, Al-Shahi Salman R, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain* 2007;130:1988-2003.
23. Poels MM, Vernooij MW, Ikram MA, Hofman A, Krestin GP, van der Lugt A, Breteler MM. Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam

scan study. *Stroke* 2010;41:S103-106.

24. 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;35:1831-1835.
25. Sveinbjornsdottir S, Sigurdsson S, Aspelund T, Kjartansson O, Eiriksdottir G, Valtysdottir B, Lopez OL, van Buchem MA, Jonsson PV, Gudnason V, Launer LJ. Cerebral microbleeds in the population based AGES-Reykjavik study: prevalence and location. *J Neurol Neurosurg Psychiatry* 2008;79:1002-1006.
26. Roob G, Schmidt R, Kapeller P, Lechner A, Hartung HP, Fazekas F. MRI evidence of past cerebral microbleeds in a healthy elderly population. *Neurology* 1999;52:991-994.
27. Horita Y, Imaizumi T, Niwa J, Yoshikawa J, Miyata K, Makabe T, Moriyama R, Kurokawa K, Mikami M, Nakamura M. [Analysis of dot-like hemosiderin spots using brain dock system]. *No Shinkei Geka.* 2003;31:263-267.
28. Qiu C, Cotch MF, Sigurdsson S, Jonsson PV, Jonsdottir MK, Sveinsbjornsdottir S, Eiriksdottir G, Klein R, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Cerebral microbleeds, retinopathy, and dementia. *Neurology* 2010;75:2221-2228.
29. 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;78:326-333.
30. Brundel M, Kwa VI, Bouvy WH, Algra A, Kappelle LJ, Biessels GJ. Cerebral microbleeds are not associated with long-term cognitive outcome in patients with transient ischemic attack or minor stroke. *Cerebrovasc Dis* 2014;37:195-202.
31. Pettersen JA, Sathiayamoorthy G, Gao FQ, 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. *Arch Neurol* 2008;65:790-795.

8

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: *J. Alzheimers Dis* 2012;31:259-263.

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 T_2*-weighted gradient echo magnetic resonance imaging (MRI), due to iron deposits thought to reflect chronic haemosiderin 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 as 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 ageing 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 artefacts 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 (M.B. and H.L.K.).[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 T_2*-weighted images (repetition time (TR)/first echo time (TE)/second TE = 20/6.9/15.8 ms, non-interpolated resolution 0.5 x 0.5 x 0.7 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 T_2*-weighted sequence (TR/TE = 1653/20 ms, non-interpolated resolution 0.99 x 0.99 x 3.00 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 (M.B. and S.M.H.) were compared and in case of discordance they performed a consensus rating. Remaining disagreements were resolved by a third observer (J.B.) (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 χ^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),

Table 1. Participant characteristics

	MCI/AD patients n=18	Controls n=18	p-value
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 3Tesla MRI, for white matter hyperintensities the total ARWMC score is shown

Table 2. Microbleed prevalence

	Any microbleeds		
	MCI/AD	Controls	p-value
7Tesla MRI			
MB prevalence (%)	14 (78)	8 (44)	0.040 *
MB No. total group	1.5 (0-80)	0 (0-5)	0.065
MB No. of MB+ group [a]	2 (1-80)	2 (1-5)	0.887
3Tesla MRI			
MB prevalence (%)	6 (33)	3 (17)	0.248
MB No. total group	0 (0-10)	0 (0-1)	0.151
MB No. of MB+ group [a]	2 (1-10)	1 (1-1)	0.039 *

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

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

* p<0.05

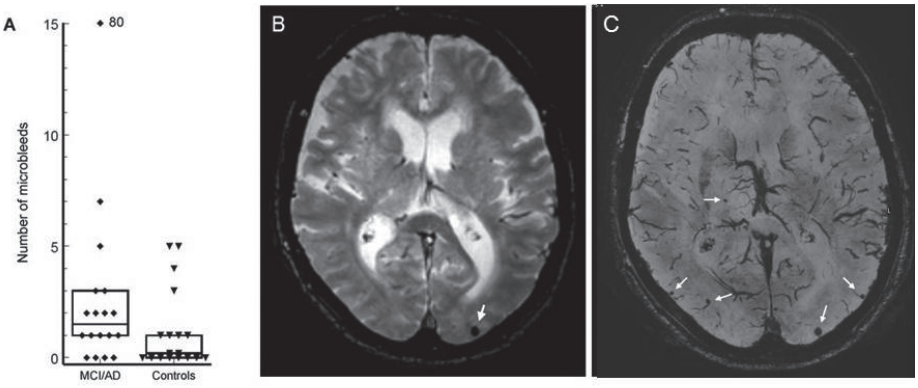


Figure 1. Total number of microbleeds at 7Tesla MRI in patients with MCI/AD and controls (box represents median with interquartile range) (A) and an example of a single slice T2*-weighted MR image at 3Tesla MRI (B) and 7Tesla MRI (second echo) (B), showing four extra microbleeds at 7Tesla MRI (arrows).

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

controls: 30 (28-30), p<0.01).

On 7Tesla 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, Fig. 1). 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 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 artefacts.

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, 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.[16,17] 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.[2,18] Moreover, because people with microbleeds, with or without a previous clinically manifest stroke, appear to be at increased risk of future haemorrhagic 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.[19]

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 aetiology, with deep and infratentorial microbleeds related to hypertensive vasculopathy and lobar microbleeds to CAA.[20,21] 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 aetiologies 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.

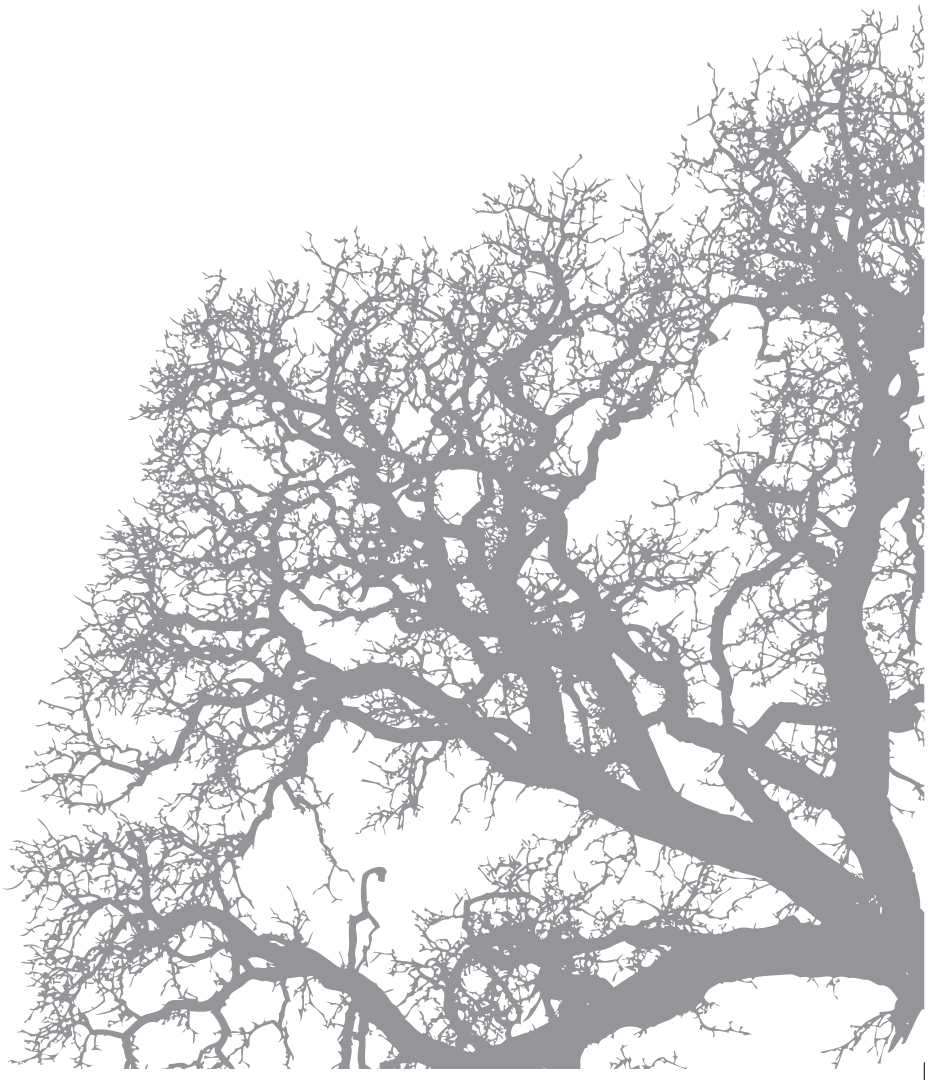
A limitation of our study is the modest sample size. This may also explain the absence of statistically significant correlations between the presence of microbleeds and age. Moreover, the stringent contra-indications for 7 Tesla MRI might have caused selection bias. However, the prevalence of microbleeds 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 aetiologies 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 Neurol* 2009;8:165-174.
2. Cordonnier C, van der Flier WM. Brain microbleeds and Alzheimer's disease: innocent observation or key player? *Brain* 2011;134:335-344.
3. Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. *J Neural Transm* 2002;109:813-836.
4. Conijn MM, Geerlings MI, Biessels GJ, Takahara T, Witkamp TD, Zwanenburg JJ, Luijten PR, Hendrikse J. Cerebral microbleeds on MR imaging: comparison between 1.5 and 7T. *AJNR Am J Neuroradiol* 2011;32:1043-1049.
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. *J Magn Reson Imaging* 2011;33:782-791.
6. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189-198.
7. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. *Br J Psychiatry* 1982;140:566-572.
8. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* 1999;56:303-308.
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;34:939-944.
10. Gregoire SM, Chaudhary UJ, Brown MM, Yousry TA, Kallis C, Jager HR, Werring DJ. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. *Neurology* 2009;73:1759-1766.
11. Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjögren M, Wallin A, Ader H, Leys D, Pantoni L, Pasquier F, Erkinjuntti T, Scheltens P; European Task Force on Age-Related White Matter Changes. A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke* 2001;32:1388-1322.
12. Yates PA, Sirisriro R, Villemagne VL, Farquharson S, Masters CL, Rowe CC. Cerebral microhemorrhage and brain {beta}-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;35:1831-1835.

14. Poels MM, Ikram MA, van der Lugt A, Hofman A, Krestin GP, Breteler MM, Vernooij MW. Incidence of cerebral microbleeds in the general population: the Rotterdam Scan Study. *Stroke* 2011;42:656-661.
15. Poels MM, Ikram MA, van der Lugt A, Hofman A, Niessen WJ, Krestin GP, Breteler MM, Vernooij MW. Cerebral microbleeds are associated with worse cognitive function: The Rotterdam Scan Study. *Neurology* 2012;78:326-333.
16. Staekenborg SS, Koedam EL, Henneman WJ, 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;40:1269-1274.
17. Kirsch W, McAuley G, Holshouser B, Petersen F, Ayaz M, Vinters HV, 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. *J Alzheimers Dis* 2009;17:599-609.
18. Sperling RA, Jack CR, Jr., Black SE, Frosch MP, Greenberg SM, Hyman BT, Scheltens P, Carrillo MC, Thies W, Bednar MM, Black RS, Brashear HR, Grundman M, Siemers ER, Feldmann HH, Schindler RJ. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: recommendations from the Alzheimer's Association Research Roundtable Workgroup. *Alzheimers Dement* 2011;7:367-385.
19. Vernooij MW, Haag MD, van der LA, Hofman A, Krestin GP, Stricker BH, Breteler MM. Use of Antithrombotic Drugs and the Presence of Cerebral Microbleeds: The Rotterdam Scan Study. *Arch Neurol* 2009;66:714-720.
20. Cullen KM, Kocsi Z, Stone J. Microvascular pathology in the aging human brain: evidence that senile plaques are sites of microhaemorrhages. *Neurobiol.Aging* 2006;27:1786-1796.
21. Fazekas F, Kleinert R, Roob G, Kleinert G, Kappeller 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. *AJNR Am.J.Neuroradiol.* 1999;20:637-642.



9 CEREBRAL MICROBLEEDS ARE NOT ASSOCIATED WITH LONG TERM COGNITIVE OUTCOME IN PATIENTS WITH TRANSIENT ISCHEMIC ATTACK OR MINOR STROKE

M Brundel

VIH Kwa

WH Bouvy

A Algra

LJ Kappelle

GJ Biessels

on behalf of the MICRO Study Group

Published in: *Cerebrovasc Dis* 2014;37:195-202.

ABSTRACT

Background Cerebral microbleeds have been related to cerebrovascular disease and dementia. They occur more frequently in patients with ischemic stroke than in the general population, but their relation with cognition in these patients is uncertain, particularly in the long run. We examined the relationship between microbleeds in patients with TIA or minor ischemic stroke, and cognitive performance 4 years later.
Methods Participants were recruited from a prospective multicentre cohort of patients with a TIA or minor ischemic stroke (n=397). They underwent Magnetic Resonance Imaging (MRI), including a T_2^*-weighted sequence, within 3 months after their ischemic event. Microbleeds, atrophy, lacunae and white matter hyperintensities (WHM) were rated visually. Cognitive status was examined in 94% of all patients who were still alive after a mean interval of 3.8 years by the Dutch version of the Telephone Interview for Cognitive Status (TICS; n=280) or by an Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) obtained from a close relative if a TICS could not be obtained (n=48). The relationship between presence of microbleeds and TICS or IQCODE was assessed with linear regression analyses adjusted for age, sex, educational level and time-interval between MRI and cognitive evaluation.
Results The mean age was 65 ± 12 years at inclusion. The vascular event at inclusion was a TIA in 170 patients (52%) and a minor ischemic stroke in 155 patients (47%). Microbleeds were present in 11.6% of the patients. Patients with microbleeds were significantly older than patients without microbleeds (70 ± 9 versus 64 ± 12 years), more often had hypertension, and had more cerebral atrophy, WMH and lacunae on MRI (all $p < 0.05$). The mean TICS score was 35.3 ± 5.9 for patients with microbleeds (n=29) and 34.6 ± 5.2 for patients without microbleeds (n=251); the adjusted mean difference (95% CI) was 1.69 (-0.01 to 3.38). The total IQCODE score was 66.0 ± 10.8 for patients with microbleeds (n= 9) and 63.1 ± 12.9 for patients without microbleeds (n=39), the adjusted mean difference was 2.43 (-7.55 to 12.41). The relative risk (adjusted for age) for abnormal cognitive performance when having microbleeds was 1.19 (95% CI: 0.63-2.26). Subcortical atrophy was associated with lower TICS score (standardized regression coefficient β: -0.12 (-0.23 to 0.00), p=0.04) and with lower IQCODE score (0.51 (0.19 to 0.83), p=0.00). The adjusted mean difference of IQCODE scores between patients with and those without a lacunar infarct was 0.39 (0.12-0.65, p=0.01).
Conclusions In this sample of patients with a recent TIA or minor ischemic stroke, microbleeds were not associated with cognitive performance 4 years later. Apparently, this association is different from other markers of small vessel disease.

INTRODUCTION

Lacunar infarcts and white matter hyperintensities (WMH) are common manifestations of cerebral small vessel disease that are linked to cognitive decline and dementia.[1] Cerebral microbleeds are another manifestation of small vessel disease that receives increasing attention. Microbleeds are visible on T2*-weighted gradient echo magnetic resonance imaging (MRI) and are primarily a radiological construct, indicative of haemosiderin deposits that are foci of past hemorrhages.[2,3] Microbleeds on MRI are common in the general population, with varying numbers reported. A systematic review including published studies from 1999 to 2004 reported a combined prevalence of 5%.[4] Microbleeds appear to be up to 4-6 times more prevalent in ischemic stroke patients[4] and patients with dementia[5] than in reference groups. Studies on the general population show a relationship between the presence of multiple microbleeds and modest cognitive deficits, mostly in the cognitive domains of executive functioning and information processing speed.[6-8] In patients who had experienced an ischemic stroke or transient ischemic attack (TIA), however, previous studies on the relation between presence of microbleeds and cognition showed inconsistent results.[9-11]

We therefore examined the relationship between the presence of microbleeds in patients with TIA or minor ischemic stroke and cognitive performance 4 years later.

METHODS

Participants

From June 2000 to January 2010 patients were prospectively included in the Microbleeds on MRI as predictors of IntraCerebral haemorrhage in patients Receiving Oral antithrombotic drugs after TIA or minor ischaemic stroke (MICRO) study, a multicentre Dutch cohort study, designed to investigate the prognostic value of microbleeds in patients using antithrombotic medication.[12] This observational study included patients presenting with a TIA or minor ischemic stroke (modified Rankin score 3 or less[13]) of presumably atherosclerotic origin, who used anticoagulants or antiplatelet agents as secondary prophylaxis. Patients with an intracranial bleed, a cerebral tumour or a history of severe head injury, or patients with a TIA or minor stroke caused by vasculitis, could not participate. Patients who were expected to die within months, with a low ability to understand or express themselves in Dutch language, or pregnancy were also excluded. Age and cognitive performance were no selection criteria.

At inclusion, information about medical history, physical and neurological examination and medication use was collected from medical records. Medical history and medication use were verified in a standardized interview by telephone. Hypertension, hypercholesterolemia and diabetes mellitus were defined as a history of this condition at baseline. A cardiovascular event was defined as a myocardial infarction or stroke, and a vascular intervention as carotid endarterectomy, coronary surgery or surgery of other arteries.

All patients underwent a brain MRI within 3 months after the ischemic event. Patients were followed up every six months by telephone, to determine whether new possible vascular events had occurred. Between September 2010 and May 2011, a cognitive screening was added to these regular telephone interviews.
The study was approved by the Institutional Review Board of all participating centres and all subjects gave written informed consent.

Brain MRI

Brain MRI was performed with 0.5- to 1.5-tesla MR scanners at the participating centers throughout the Netherlands. All MRI protocols included a T2*-weighted fast field echo (FFE) gradient echo sequence. The MRI sequence parameters that were used are provided in online supplementary table 1. Scans were evaluated by standardized visual rating scales at one investigational center.

Microbleeds were visually scored according to the Microbleed Anatomical Rating Scale (MARS), an instrument with good intra- and interrater reliability for the presence of definite microbleeds.[14] Microbleeds were scored separately for different brain regions (i.e. infratentorial, deep and lobar).

Brain infarcts were scored as large vessel infarcts (>1.5 cm) or lacunar infarcts. Lacunar infarcts were defined as a focal lesion <1.5 cm in diameter, with a hypointense core on T1 and FLAIR images and with an appearance unlike a perivascular space.

Cortical atrophy was measured semi-quantitatively with two ratios: the frontal interhemispheric fissure ratio and the Sylvian fissure ratio.[15] Subcortical atrophy was evaluated by the bicaudate ratio.[15] The raw scores were standardised into z scores based on the mean score of all subjects (the z score of cortical atrophy was derived by calculating the mean of de frontal interhemispheric ratio and the Sylvian fissure ratio). WMH were scored according to the Age-Related White Matter Changes (ARWMC) scale.[16]

Each MRI scan was independently rated by 2 observers who were blinded to clinical information. In case of disagreement for microbleed or cerebral infarct ratings, consensus was obtained in a consensus meeting (intraclass correlation

coefficient for number of microbleeds: 0.70 (0.64-0.75)). For WMH and atrophy ratios, the mean score of both observers was used.

Cognitive assessment

The cognitive status of all patients who were alive and could be contacted was examined with the Dutch version of the Telephone Interview for Cognitive Status (TICS),[17] a 12-item screening instrument designed to identify persons with dementia.[18] A TICS score of 26 or lower was considered abnormal.[17] Depressive symptoms were assessed by the Dutch version of the Mini International Neuropsychiatric Interview, a brief screening instrument for the presence of major depression.[19]

For 48 patients who could be traced and were still alive at follow-up, a TICS could not be obtained due to deafness (n=9), communication problems (aphasia, severe dysarthria, or not native Dutch speaker (n=11)), admission to a hospital or nursing home (n=10), dementia (n=7), refusing TICS (n=3), absence of the patient (n=4) or severe fatigue or illness (n=4). In these 48 cases, the short version of the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE, Dutch version) was obtained from a close relative (mostly a partner). The IQCODE is a 16-item screening test which assesses cognitive change in everyday activities of patients; a higher score means greater cognitive decline from a previous level. A mean IQCODE of 3.6 or higher was considered abnormal.[20]

Statistical analysis

Between-group differences in subject characteristics were analyzed by analysis of variance for continuous variables, Mann-Whitney U tests for nonparametric data and χ^2 tests for proportions. The relationships between the presence of microbleeds and cognitive performance (groups with a TICS and an IQCODE score separately) were examined by linear regression analyses, adjusted for age, sex, time interval between MRI and cognitive screening, and depression. For the association between microbleeds and TICS score, analyses were also adjusted for educational level (because the IQCODE assesses change in cognition, no adjustment for education level is needed). Poisson regression analyses were used to assess the risk of abnormal cognition (i.e. TICS score ≤26 or mean IQCODE ≥3.6), both unadjusted and adjusted for age, sex, time interval between MRI and cognitive screening, education level and depression.

We performed a power calculation, under the assumption of a microbleed prevalence of 16% in this patient group (α = 0.05 and β = 0.2). Based on these assumptions, we needed 154 patients with, and 26 patients without microbleeds to detect a difference of 3 points on the TICS (95% CI 0.9-5.1).

RESULTS

In total, 397 patients with a brain MRI were included in the MICRO study. By the time of cognitive follow up, 47 patients had died. At inclusion these latter patients were significantly older than the 350 survivors (75.1 ± 9.7 vs. 64.6 ± 11.9 years), had a higher modified Rankin Scale score (median of 2 vs. 1) and more often had microbleeds on MRI (21% vs. 11%). Of the surviving 350 patients, cognitive follow up was obtained for 328 patients (94%); 15 patients were not willing to participate and 7 patients could not be contacted (Figure 1). Patients alive without cognitive follow up (n=22) did not differ from patients with cognitive follow up (n=328) with regard to baseline sex, age, vascular event at inclusion and modified Rankin Scale score. None of them showed microbleeds on MRI.

The baseline characteristics of the study population are shown in table 1. The mean age of the patients was 65 ± 12 years (range 28-88 years), and 138 patients (42%) were male. The vascular event at inclusion was a TIA in 170 patients (52%), a minor ischemic stroke in 155 patients (47%), and unknown in 3 patients. One or more microbleeds were found in 38 patients (11.6%): in 19 patients with a minor ischemic stroke (12.3%) and in 18 patients with a TIA (10.6%). Patients with microbleeds did not differ from patients without microbleeds in sex (40% vs. 42% male; $p > 0.05$), but they were significantly older (70 ± 9 vs. 64 ± 12 years; $p < 0.01$). The vascular risk factor profile was comparable between the two groups, except for a higher systolic blood pressure and more patients with a history of hypertension in the group with microbleeds (both $p < 0.05$; table 1).

Brain MRI findings of patients with and those without cerebral microbleeds are shown in table 2. Of the 38 patients with microbleeds, 12 patients (32%) had multiple microbleeds, with a maximum of 33 microbleeds in 1 patient. Deep microbleeds were found in 17 patients (45%), infratentorial microbleeds in 12 patients (32%) and lobar microbleeds in 21 patients (55%), of which most frequently in the parietal lobe (n=9; 24%), followed by the frontal lobe (n=7; 18%), occipital lobe (n=6; 16%) and temporal lobe (n=3; 8%). Strictly deep/infratentorial and strictly lobar microbleeds occurred in 45% and 40% of the patients, respectively. Both cortical and subcortical atrophy were relatively more pronounced in patients with microbleeds ($p < 0.05$). Furthermore, presence of microbleeds was clearly associated with other markers of small vessel disease (lacunar infarcts and WMH; both $p < 0.05$).

On average, cognition was measured 3.8 ± 2.5 years after MRI. Patients assessed by the IQCODE were significantly older than those assessed by the TICS (72 ± 11 years for the IQCODE group vs. 64 ± 12 for the TICS group). A total of 14% of patients

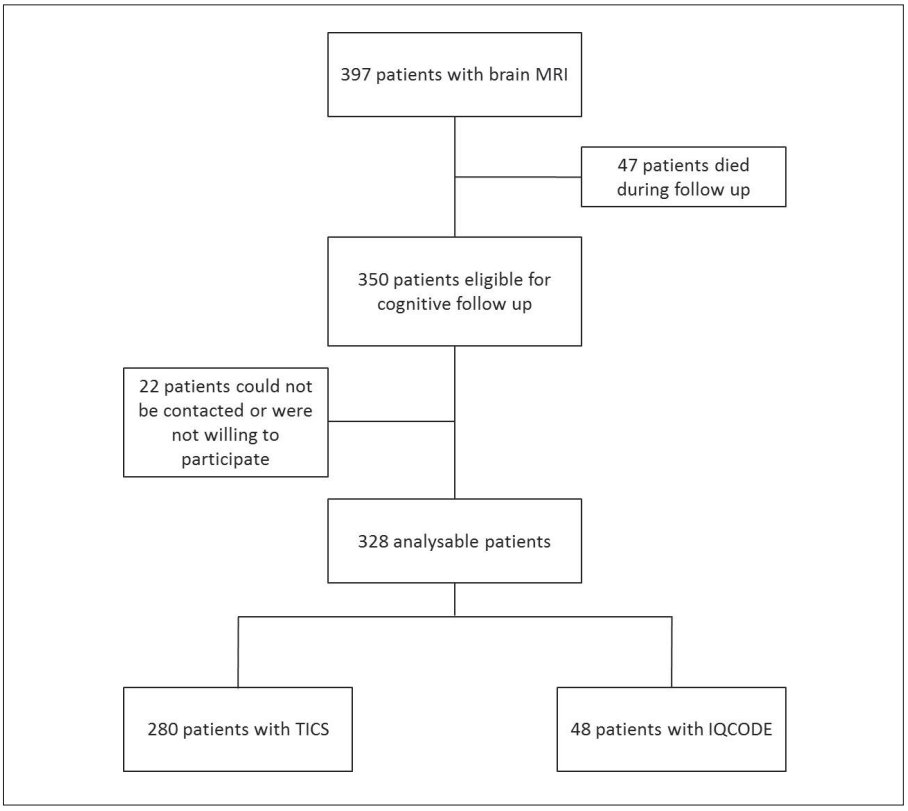


Figure 1. Flow chart of the study

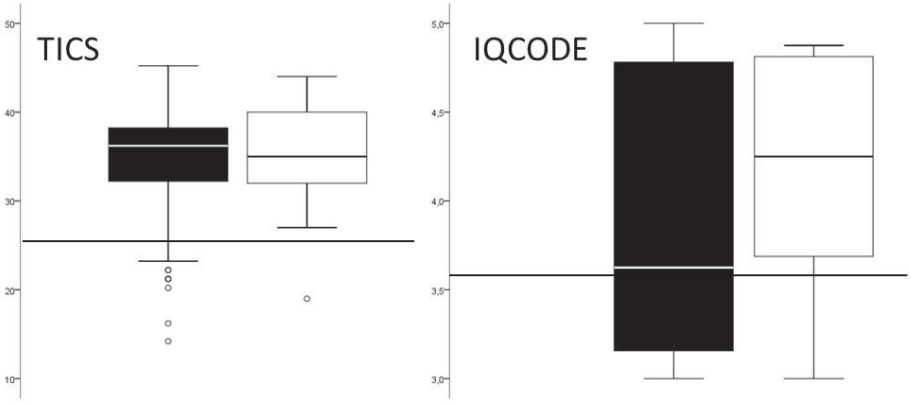


Figure 2. Total TICS and mean IQCODE scores for patients without (■) and with (□) microbleeds. The line represents the cut-off for abnormal cognition: for TICS a score ≤ 26 is considered abnormal, for IQCODE a mean score ≥ 3.6.

Table 1. Patient characteristics at inclusion

	No microbleeds n = 290	≥1 Microbleed n = 38	p-value
Sex (male)	122 (42)	15 (41)	0.85
Age (years, mean ± SD)	64 ± 12	70 ± 9	0.00 *
Education level	4.8 ± 1.4	4.7 ± 1.5	0.70
TIA at inclusion	152 (52)	18 (47)	0.45
Ischemic stroke at inclusion	136 (47)	19 (50)	0.60
Modified Rankin scale (median; IQR)	1; 0-1	1; 0-1	0.38
Modified Rankin scale 0	135 (47)	14 (37)	
Modified Rankin scale 1	90 (31)	15 (40)	
Modified Rankin scale ≥ 2	61 (21)	9 (24)	
Time interval MRI – cognitive screening	3.8 ± 2.5	3.9 ± 2.5	0.74
Mean systolic blood pressure, mm Hg (n=256)	153 ± 28	161 ± 30	0.05 *
Mean diastolic blood pressure, mm Hg	87 ±16	88 ± 14	0.78
Hypertension [a]	156 (54)	29 (76)	0.02 *
Hypercholesterolemia [a]	126 (43)	18 (47)	0.53
Diabetes mellitus [a]	36 (12)	4 (11)	0.82
Current smoking	78 (27)	8 (21)	0.56
History of cardiovascular event [b]	36 (12)	5 (13)	0.86
Antiplatelet use	59 (20)	11 (29)	0.23
Anticoagulant use	5 (2)	0 (0)	0.42

Data presented as n (%) unless otherwise specified

[a] Hypertension, hypercholesterolemia and diabetes mellitus were defined as a history of this condition at baseline.

[b] Cardiovascular event was defined as a myocardial infarction or stroke

* $p < 0.05$

Table 2. MRI characteristics

	No microbleeds n = 290	≥1 Microbleed n = 38	p-value
Microbleeds			
Microbleed No. total (median, range)	-	1 (1-33)	-
Microbleed No. deep/infratentorial (median, range)	-	1 (0-7)	-
Microbleed No. lobar (median, range)	-	1 (0-26)	-
Multiple microbleeds	-	12 (32)	-
Strictly deep/infratentorial microbleeds	-	17 (45)	-
Strictly lobar microbleeds	-	15 (40)	-
Other MRI measures			
Cortical atrophy z score [a]	-0.08 ± 0.97	0.48 ± 1.10	0.00 *
Subcortical atrophy z score [b]	-0.11 ± 0.96	0.38 ± 1.13	0.00 *
Lacunar infarct	92 (32)	23 (61)	0.00 *
Large vessel infarct	86 (30)	11 (29)	0.10
ARWMC total score [c]	4.7 ± 4.3	10.5 ± 6.8	0.00 *

Data presented as mean ± standard deviation or proportions (%) unless otherwise specified

[a] Cortical atrophy: z score of the mean of the frontal fissure ratio and the Sylvian fissure ratio

[b] Subcortical atrophy: z score of the bicaudate ratio

[c] Age-related White Matter Changes score[15]

* $p < 0.05$

scored below the cut off value for either test. The mean TICS score was 35.3 ± 5.9 for patients with microbleeds (n=29) and 34.6 ± 5.2 for patients without microbleeds (n=251); the adjusted mean difference (95% CI) was 1.69 (-0.01; 3.38; p=0.05; a higher TICS score reflects better cognitive performance). The total IQCODE score was 66.0 ± 10.8 for patients with microbleeds (n= 9) and 63.1 ± 12.9 for patients without microbleeds (n=39); the adjusted mean difference was 2.43 (-7.55; 12.41; p=0.62; a higher IQCODE score reflects worse cognitive performance; figure 2). Comparable results were found when patients with multiple (≥2) microbleeds were compared with patients without microbleeds (adjusted mean difference in TICS score: -0.87 (-3.79; 2.04), p=0.56; adjusted mean difference in IQCODE score 5.31 (-9.58; 20.19; p=0.48). The unadjusted risk ratio for abnormal cognitive performance when having microbleeds was 1.57 (95% CI 0.79-3.09). The relative risk adjusted for age

Table 3. List of publications reporting the relationship between microbleeds and cognitive functioning in patients with cerebrovascular disease

Authors	Patient group	No. of patients	No. of patients with MBs (prevalence)
Werring et al. (2004) [10]	Neurovascular clinic	55	25 [a]
Gregoire et al. (2012) [27]	Neurovascular clinic	26	9 [a]
Gregoire et al. (2013) [9]	TIA or ischemic stroke	320	72 (23)
Patel et al. (2013) [11]	Lacunar infarct and WMH	116	46 (40)
Zhang et al. (2013) [26]	Lacunar infarct	85	35 (41)

MB = microbleed; CVA = cerebrovascular accident, i.e. TIA or ischemic stroke; NPA = neuropsychological assessment; x = unknown

[a] No microbleed prevalence is given because this was a case-control study

[b] Montreal Cognitive Assessment (MoCA) questionnaire

was 1.19 (0.63-2.26). Moreover, the presence of microbleeds was not associated with an indication of depression according to the Mini International Neuropsychiatric Interview (standardized regression coefficient β: -0.05 (-0.15; 0.07; p=0.43).

In contrast to the findings for microbleeds, other MRI measures of small vessel disease and atrophy were found to be associated with cognition 4 years later. Subcortical atrophy was associated with TICS score (standardized regression coefficient β (adjusted for age, sex, educational level and time-interval between MRI and cognitive assessment): -0.12 (-0.23; 0.00); p=0.04) and IQCODE score (0.51 (0.19; 0.83); p=0.00). Presence of lacunar infarcts was associated with IQCODE score (adjusted mean difference of IQCODE score between patients with and those without a lacunar infarct: 0.39 (0.12; 0.65); p=0.01). For cortical atrophy, these associations did not reach statistical significance (standardized β for TICS score: -0.11 (-0.23; 0.00); p=0.06; standardized β for IQCODE score: 0.07 (-0.01; 0.15); p=0.07. WMH were not associated with cognition.

Cognition	Time-interval CVA and NPA	Association between microbleeds and cognition
Extensive NPA	0 months	Presence of MBs - executive impairment (no relation with general cognitive functioning or 4 other cognitive domains)
Extensive NPA	5.7 years	Presence of MBs – executive impairment (no relation with general cognitive functioning or other cognitive domains)
Extensive NPA	<3 months	Presence/number of strictly lobar MBs – executive impairment (no relation with global cognitive performance or 5 other cognitive domains; no relation between other MB locations and cognitive performance)
4 cognitive domains	>3 months (mean: 2.9 years)	≥9 MBs – executive impairment (no relation between presence/number of MBs and 4 cognitive domains)
MoCA b	x	Presence of MBs – global cognitive impairment

DISCUSSION

In this cohort of 328 patients with a TIA or minor ischemic stroke, global cognitive performance after 4 years did not differ between patients with and those without microbleeds.

Strengths of our study include its sample size and the fact that cognitive follow-up was obtained from 94% of the survivors after multiple years. The cohort entailed patients with a recent TIA or minor ischemic stroke who underwent brain MRI and to whom antithrombotic medication was prescribed from multiple centers throughout the Netherlands. This patient group is representative of patients with a TIA or minor ischemic stroke in a regular vascular outpatient clinic. We chose to assess cognition by telephone to ensure a high follow-up rate, thus limiting attrition. The downside of this approach is that a full face-to-face cognitive assessment by a trained neuropsychologist obviously provides more details on cognition than the TICS or the IQCODE. Nevertheless, the TICS has previously been shown to correlate fairly well with multiple cognitive domains on a comprehensive neuropsychological assessment.21 Because we addressed global cognitive performance, a relationship

with specific cognitive domains could have been overlooked. Though, the fact that brain atrophy and lacunae were associated with worse cognitive performance in our cohort, supports the validity of our approach and is in line with earlier studies showing similar associations in patients with ischemic stroke[22] or subcortical ischemic vascular disease.[23] Since the 47 patients who had died at the time of cognitive follow-up significantly more often showed microbleeds on MRI, our results might be influenced by selection bias. However, in post-hoc analysis, with the combination of death or abnormal cognition as the endpoint, the risk ratio did not change significantly (data not shown). Furthermore, we did not intend to study the prediction of cognitive impairment in the long run, but specifically addressed the question whether microbleeds are a long-term determinant of cognitive performance, i.e. in the survivors.

A limitation of our study is that different MR scanners were used. Still, field strength was 1.5 T in 77% of the patients, and in only 4.8% of the patients, 0.5 T was used; 1.5 T MRI is still widely used in current daily practice. Nevertheless, field strength and older scan acquisition protocols may explain the relatively low microbleed prevalence of 11.6% in our study compared with that in previously published papers (a mean prevalence of 21.5% was reported in a meta-analysis).[4] However, we did not find any indication of a relationship between the presence of microbleeds and cognition in our study sample. Therefore, we do not expect that the use of a more sensitive MRI technique would essentially change our results.

Increasing numbers of papers point to the potential clinical relevance of microbleeds. In patients with a past ischemic event, the presence of cerebral microbleeds has been associated with future cerebrovascular accidents.[4,12,24] This topic is especially important to address in the light of antithrombotic therapy.[25] Cognition has been identified as another potential clinical correlate of microbleeds. Five other studies have investigated microbleeds as risk factor for cognitive impairment in patient with TIA or ischemic stroke (overview in table 3).[9-11,26,27] Associations between the presence of microbleeds and cognitive performance were noted, but this was mostly restricted to decrements in executive functioning and did not extend to other domains. Moreover, associations with cognition were mainly observed for specific subgroups of patients with microbleeds, particularly those with lobar or multiple microbleeds. Most of these earlier studies assessed cognition within the first months after stroke. However, cognitive performance deficits can improve during the first months after stroke and in a significant proportion of patients cognitive deficits are temporary.[28] One study found a relation between microbleeds and cognition only in a small subgroup of patients (n=12) with multiple microbleeds after a mean

follow up of 2.9 years, whereas there was no relationship for the whole population of 116 patients.[11] Another study reported an association between the presence of microbleeds at baseline and executive impairment after a mean follow up of 5.7 years in only nine patients, but the analyses were not adjusted.[27]

Previous studies on large population-based cohorts reported associations between cerebral microbleeds and cognitive performance.[6,8] Nevertheless, the magnitude of the microbleed-associated cognitive decrements is small, for example 0.4 MMSE points, or z-scores on cognitive domains ranging from 0 to 0.4, for people with 5 or more microbleeds compared to those without microbleeds in fully adjusted models.[8] The difference to the results for patients with a TIA or ischemic stroke may partly be explained by the fact that microbleeds are markers of different pathological processes, e.g. arteriolosclerosis or amyloid angiopathy. In different study populations, different pathologies will dominate. In stroke populations, many people will have arteriolosclerosis and related forms of small vessel disease, also patients without microbleeds. By comparison, in a population based setting the overall burden of vascular pathology, both related to arteriolosclerosis and amyloid angiopathy, will be lower, which may increase the contrast between people with and without MRI markers of small vessel disease such as microbleeds.

Although it can be argued that we did not use the most sensitive techniques to assess the presence of microbleeds and cognitive performance, it should be noted that we did not find any indication of a relationship with cognitive performance in our microbleed-positive subjects. The point estimates for differences between the groups were close to zero or even tended to favour the microbleed-positive group. Therefore we can conclude that there does not appear to be a clinically meaningful relationship between microbleeds and cognition in the long run in a general stroke population. Apparently, this association is different from other markers of small vessel disease.

Supplementary table 1. T2*W FFE transversal MRI parameters used in the different centers

	UMCU	UMCU	Slotervaart	MST	Antonius	Meander
magnet field strength	0.5T	1.5T	1.0T	1.5T	1.5T	1.0T
number of slices	19	20	22	20	22	20
slice thickness (mm)	6.0	6.0	5.0	6.0	5.0	6.0
slice gap (mm)	1.2	1.2	1.0	1.2	1.0	0.6
echoes	1	1	1	1	1	1
TE (ms)	27	23	21	27	23	18
flip angle	15	20	15	15	-	20
TR (ms)	848	663	680	847	668	612

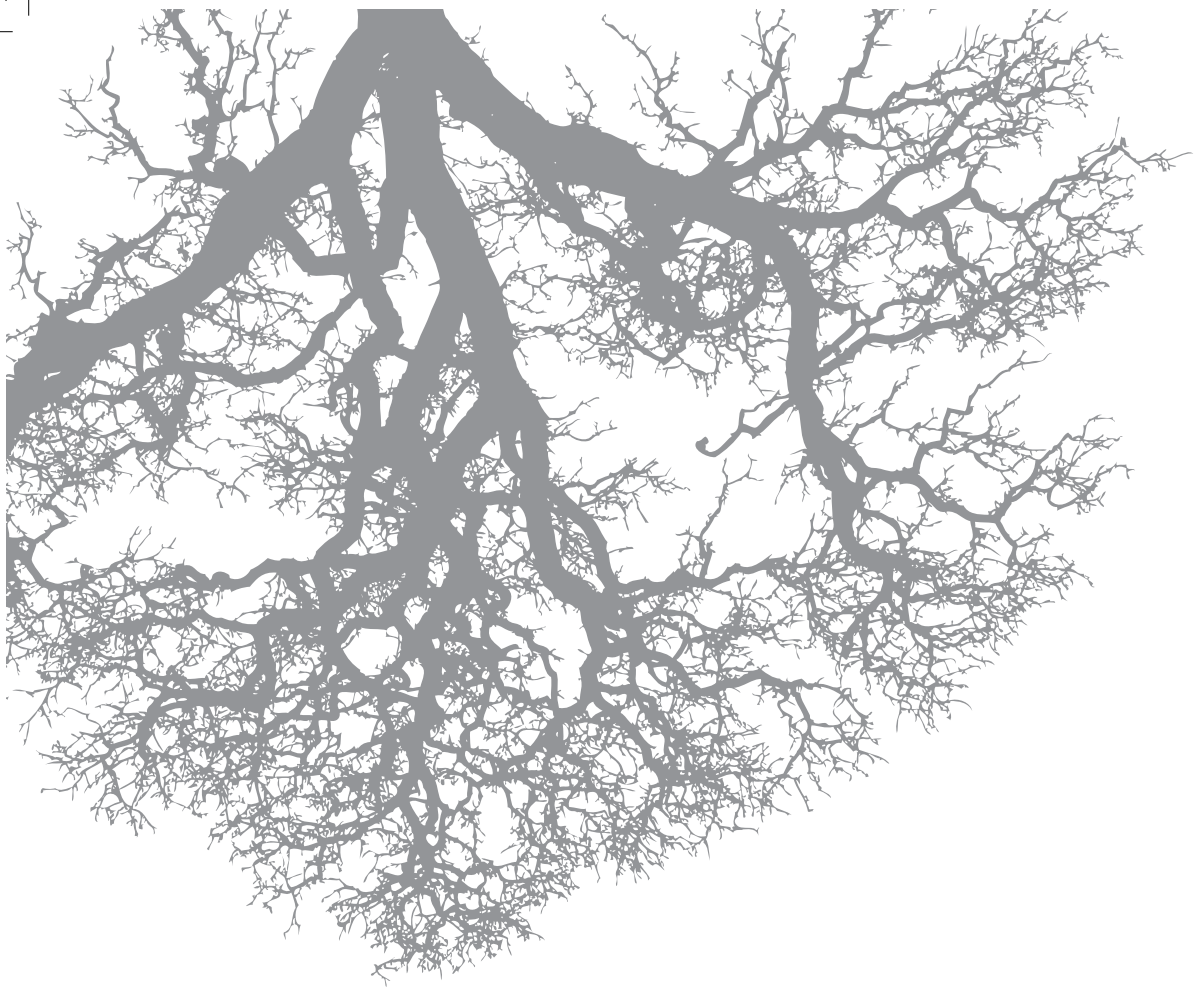
- = unknown

REFERENCES

1. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* 2010;9:689-701.
2. 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 Neurol* 2009;8:165-174.
3. Shoamanesh A, Kwok CS, Benavente O. Cerebral microbleeds: histopathological correlation of neuroimaging. *Cerebrovascular Dis* 2011;32:528-534.
4. Cordonnier C, Al-Shahi Salman R, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain* 2007;130:1988-2003.
5. Cordonnier C, van der Flier WM: Brain microbleeds and Alzheimer's disease. innocent observation or key player? *Brain* 2011;134:335-344.
6. Qiu C, Cotch MF, Sigurdsson S, Jonsson PV, Jonsdottir MK, Sveinbjrnsdottir S, Eiriksdottir G, Klein R, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. *Neurology* 2010;75:2221-2228.
7. Pettersen JA, Sathiyamoorthy G, Gao FQ, 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. *Arch Neurol* 2008;65:790-795.
8. Poels MM, Ikram MA, van der Lugt A, Hofman A, Niessen WJ, Krestin GP, Breteler MM, Vernooij MW. Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. *Neurology* 2012 ;78:326-333.
9. Gregoire SM, Scheffler G, Jager 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* 2013;44:1267-1272.
10. Werring DJ, Frazer DW, Coward LJ, Losseff NA, Watt H, Cipolotti L, Brown MM, Jager HR. Cognitive dysfunction in patients with cerebral microbleeds on T2*-weighted gradient-echo MRI. *Brain* 2004;127:2265-2275.
11. Patel B, Lawrence AJ, Chung AW, Rich P, Mackinnon AD, Morris RG, Barrick TR, Markus HS. Cerebral microbleeds and cognition in patients with symptomatic small vessel disease. *Stroke* 2013;44:356-361.
12. Kwa VI, Algra A, Brundel M, Bouvy W, Kappelle LJ. Microbleeds as a predictor of intracerebral haemorrhage and ischaemic stroke after a TIA or minor ischaemic stroke: a cohort study. *BMJ Open* 2013;3:e002575.

13. Rankin J. Cerebral vascular accidents in patients over the age of 60. II. Prognosis. *Scott Med J* 1957;2:200-215.
14. Gregoire SM, Chaudhary UJ, Brown MM, Yousry TA, Kallis C, Jager HR, Werring DJ. The Microbleed Anatomical Rating Scale (MARS): reliability of a tool to map brain microbleeds. *Neurology* 2009;73:1759-1766.
15. Gomori JM, Steiner I, Melamed E, Cooper G. The assessment of changes in brain volume using combined linear measurements. A CT-scan study. *Neuroradiology* 1984;26:21-24.
16. 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, European Task Force on Age-Related White Matter Changes. A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke* 2001;32:1318-1322.
17. Kempen GI, Meier AJ, Bouwens SF, van DJ, Verhey FR. [The psychometric properties of the Dutch version of the Telephone Interview Cognitive Status (TICS)]. *Tijdschr Gerontol Geriatr* 2007;38:38-45.
18. Brandt J, Spencer M, Folstein M. The Telephone Interview for Cognitive Status. *Cogn Beh Neurol* 1988;1:111-117.
19. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 1998;59:22-33.
20. De Jonghe JF, Schmand B, Ooms ME, Ribbe MW. [Abbreviated form of the Informant Questionnaire on cognitive decline in the elderly]. *Tijdschr Gerontol Geriatr* 1997;28:224-229.
21. Van den Berg E, Ruis C, Biessels GJ, Kappelle LJ, Van Zandvoort MJ. The Telephone Interview for Cognitive Status (Modified): relation with a comprehensive neuropsychological assessment. *J Clin Exp Neuropsychol* 2012;34:598-605.
22. Stebbins GT, Nyenhuis DL, Wang C, Cox JL, Freels S, Bangen K, de Toledo-Morrell L, Sripathirathan K, Moseley M, Turner DA, Gabrieli JD, Gorelick PB. Gray matter atrophy in patients with ischemic stroke with cognitive impairment. *Stroke* 2008;39:785-793.
23. Fein G, Di Sclafani V, Tanabe J, Cardenas V, Weiner MW, Jagust WJ, Reed BR, Norman D, Schuff N, Kusdra L, Greenfield T, Chui H. Hippocampal and cortical atrophy predict dementia in subcortical ischemic vascular disease. *Neurology* 2000;55:1626-1635.
24. Fan YH, Zhang L, Lam WW, Mok VC, Wong KS. Cerebral microbleeds as a risk factor for subsequent intracerebral hemorrhages among patients with acute ischemic stroke. *Stroke* 2003;34:2459-2462.
25. Soo YO, Yang SR, Lam WW, Wong A, Fan YH, Leung HH, Chan AY, Leung C, Leung TW, Wong LK. Risk vs benefit of anti-thrombotic therapy in ischaemic stroke patients with cerebral microbleeds. *J Neurol* 2008;255:1679-1686.

26. Zhang M, Chen M, Wang Q, Yun W, Zhang Z, Yin Q, Huang Q, Zhu W. Relationship between cerebral microbleeds and cognitive function in lacunar infarct. *J Int Med Res* 2013;41:347-355.
27. Gregoire SM, Smith K, Jager HR, Benjamin M, Kallis C, Brown MM, Cipolotti L, Werring DJ. Cerebral microbleeds and long-term cogntive outcome: longitudinal cohort study of stroke clinic patients. *Cerebrovasc Dis* 2012;33:430-435.
28. Nys GM, Van Zandvoort MJ, de Kort PL, Van der Worp HB, Jansen BP, Algra A, de Haan EH, Kappelle LJ. The prognostic value of domain-specific cognitive abilities in acute first-ever stroke. *Neurology* 2005;64:821-827.



10 CEREBRAL MICROBLEEDS ARE NOT RELATED TO COGNITION IN PATIENTS WITH A TIA OR ISCHEMIC STROKE

M Brundel

AG van der Kolk

SM Heringa

PR Luijten

LJ Kappelle

J Hendrikse

GJ Biessels

ABSTRACT

Background Cognitive impairment frequently occurs after stroke. This can be the result of the stroke itself, but it can also be caused by concomitant cerebrovascular disease, in particular small vessel disease (SVD). Microbleeds are one of the MRI features of SVD. It is uncertain if microbleeds are related to the cognitive performance of patients who have suffered from an ischemic stroke. We investigated the association between microbleeds at 7T MRI and cognitive performance in patients with ischemic stroke or transient ischemic attack (TIA).

Methods Patients with partial or total anterior circulation infarction or TIA were eligible for this study. Forty-four patients (mean age 60.2 ± 14.3 years, 61% male) were included. They all underwent 7T MRI, including a T2*-weighted and a FLAIR sequence, and a detailed neuropsychological assessment. Microbleeds, brain atrophy, brain infarcts and white matter hyperintensities (WMH) were rated visually. The relationship between the presence of microbleeds and cognitive performance was examined with linear regression analyses.

Results Twenty-four (55%) patients had an ischemic stroke and 20 (46%) patients had a TIA. Microbleeds were found in 15 patients (34%). Patients with and without microbleeds did not differ in age, sex, type of the vascular event, or vascular risk factor profile. Patients with microbleeds more often had lacunar infarcts (47% vs 7%, $p<0.05$); WMH and brain atrophy did not differ between the groups. Patients with microbleeds performed slightly worse on all four cognitive domains compared with those without, but differences were not statistically significant (adjusted mean difference for composite cognition in standardized z-scores (95% CI): -0.16 (-0.67; 0.36)).

Conclusion In patients with ischemic stroke or TIA of the anterior circulation, microbleeds as detected on 7T MRI did not contribute to cognitive impairment.

INTRODUCTION

Stroke is a major cause of death and disability. It often leads to cognitive dysfunction,[1] and doubles the risk of developing dementia,[2] independent of its aetiology.[1,3] Cognitive dysfunction can be caused by the stroke itself, but it can also be caused by concomitant cerebrovascular disease, in particular small vessel disease (SVD). SVD is a disorder affecting the small perforating vessels in the brain.[4] Lacunar infarcts and white matter hyperintensities (WMH) are frequently observed markers of SVD on magnetic resonance imaging (MRI).[5] Cerebral microbleeds, visible as small black dots on T2*-weighted MR sequences, receive increasing attention as an additional imaging marker of SVD.[5] They are common in the general population,[6,7] and seem to be up to 4-6 times more prevalent in patients with ischemic stroke[6] and in patients with dementia.[8]

In community-dwelling elderly, the presence of microbleeds has been related to mild cognitive deficits, mostly in the domains attention and executive functioning, and information processing speed.[9,10] In patients with transient ischemic attack (TIA) or ischemic stroke, however, studies report inconsistent results.[11-13] Noticeably, in these patients, the association between microbleeds and cognitive performances in patients suffering from stroke or TIA has thus far only been investigated at regular (1-3 tesla (T)) MR field strength. It is known that the detection of microbleeds is highly dependent on scan protocol and magnetic field strength. At ultra-high field 7T MRI, the higher attainable spatial resolution, increased contrast-to-noise-ratio, and increased susceptibility effect, have resulted in a three times higher number of detected microbleeds compared with 1.5T MRI.[14,15] Associations with clinical outcomes, such as cognitive performance, could differ with a better detection of microbleeds.

Therefore, the present study examined in patients with partial or total anterior circulation infarction or TIA, whether additional microbleeds at 7T MRI as a marker of concomitant SVD, is related to cognitive performance.

METHODS

Study population and procedures

Patients were included as part of a study on intracranial vessel wall imaging.[16] Between December 2009 and October 2012, patients who presented at the neurology ward of our institution with arterial ischemic stroke or TIA in the anterior cerebral circulation, were screened. Patients with an acute ischemic stroke were included in case of a Total Anterior Circulation Infarct (TACI) or Partial Anterior Circulation

Infarct (PACI).[17] In patients with a TIA it can be more difficult to distinguish between a lacunar syndrome or partial or total anterior circulation syndrome, because the symptoms have often disappeared at presentation in the hospital. Therefore, all TIA patients with symptoms that were attributable to the anterior circulation were eligible. Exclusion criteria were: 1. inability to undergo the MRI exam within one week due to clinical condition; 2. contraindications for 7T MRI (claustrophobia, metallic objects in or on the body); 3. a known allergic reaction to gadolinium contrast media or impaired renal function (a gadolinium-based contrast agent was used for the vessel wall sequence). 7T MR scanning was performed within one week of symptom onset and repeated approximately one month after symptom onset. A neuropsychological assessment was performed, generally on the day of the second MRI scan.

Inclusion criteria for the present study were availability of 1. a T2*-weighted MRI sequence (either at the first or second MR exam) and 2. a neuropsychological examination. Patients with a pre-existent psychiatric or neurological disorder that could affect cognitive functioning were excluded. A prior stroke or TIA was no exclusion criterion. Of 58 included patients who underwent 7T MRI, 3 had insufficient quality of the T2*-weighted sequence, and from 11 patients no neuropsychological assessment data were available. Thus, for the present study, 44 patients were eligible.

The study was approved by the institutional review board of our institution, and all subjects gave written informed consent.

MRI protocol

Imaging was performed on a 7T whole-body MR system (Philips Healthcare, Cleveland, OH, USA) with a 16- or 32-channel receive coil and a volume transmit/receive coil for transmission (Nova Medical, Wilmington, MA). The standardized protocol included a triple-echo T2*-weighted sequence (repetition time (TR)/echo time (TE) = 22/2.5;9.8;17.1 ms, acquired voxel size 0.40 x 0.50 x 0.60 mm^3) and a fluid-attenuated inversion recovery (FLAIR) sequence (TR/inversion time (TI)/TE: 8107/2415/294 ms, acquired voxel size 0.99 x 1.00 x 1.10 mm^3).

Analyses were done on the MRI scan that was performed within one week after the vascular event. If this scan was of insufficient quality the follow-up MR scan was used.

Detection of microbleeds

Microbleeds were detected on the T2*-weighted images with the previously described semi-automatic method based on the radial symmetry transform (RST).[18] For the RST the second and third echo of the T2*-weighted sequence were used. The method

was slightly modified by incorporating minimum intensity projection images. This improves sensitivity and reduces the number of suspected microbleed locations.[19] The RST result was censored visually to select true microbleeds by two independent raters, blinded to clinical information. In case of disagreement, consensus was obtained in a consensus meeting.

The inter-rater agreement was good for the number of microbleeds (intra-class coefficient (ICC) (95% CI): 0.84 (0.73-0.91)).

Other MRI markers

WMH, cerebral infarcts and brain atrophy were rated visually on FLAIR images. WMH load was scored according to the Age-Related White Matter Changes (ARWMC) scale.[20] Only supratentorial WMH were assessed because inhomogeneity in the applied transmit field at 7T, which is most prominent infratentorially, can lead to contrast deviation. Consequently, the maximum ARWMC score was 24. The presence and number of both lacunar (<1.5 cm) and large vessel infarcts were rated. Cortical and subcortical atrophy were measured semi quantitatively with a scale (0-3) based on the width of the Sylvian and interhemispheric fissure, and the size of the frontal horns and lateral ventricles, respectively.

All scans were independently rated by two observers blinded to clinical information. In case of disagreement regarding infarct ratings, consensus was obtained in a consensus meeting. For WMH and atrophy scores, the mean score of both observers was used.

Cognitive testing

All participants underwent a detailed standardized cognitive assessment at least one month after the vascular event. In four patients, cognitive testing was performed within one week after the symptom onset, for practical reasons. Global cognitive functioning was assessed by the Mini Mental State Examination (MMSE). In addition, ten tasks were administered, divided into four cognitive domains to reduce the amount of neuropsychological variables. The domain memory was assessed by the immediate and delayed task of the Rey Auditory Verbal Learning Test, the Rey Complex Figure Test (delayed) and the forward and backward digit span of the Wechsler Adult Intelligence Scale-III (WAIS-III). The domain language was assessed by the Boston Naming Task and the Token test. The domain attention and executive functioning was assessed by the Rule Shift Test, the Ruff figural fluency test and a letter fluency test using the 'N' and 'A'; and category fluency (animal naming). The domain visuoconstruction and –perception was assessed by the

Rey Complex Figure Test, the Face Recognition Test and the Judgement of Line Orientation test.

For each cognitive test, raw test scores were standardized into z-scores, by using the pooled mean of the whole group, and averaged to obtain one composite z-score per cognitive domain. These z-scores per domain were averaged to obtain one composite z-score.

Statistical analyses

Patient characteristics were analysed with analysis of variance for continuous variables, Mann-Whitney U tests for non-parametric data and χ^2 tests for proportions. Comparisons were made between patients with versus patients without microbleeds. After post-hoc inspection of the microbleed frequency distribution, we enhanced contrast by comparing patients with no versus patients with three or more microbleeds. The relationship between the presence of microbleeds and cognitive performance was examined with linear regression analyses, adjusted for age and sex, and in the case of a significant association additionally for education level (no adjustment for other variables was made, because of the modest sample size and because they did not differ between patients with and without microbleeds).

RESULTS

Patient characteristics are shown in table 1. Forty-four patients were included. Mean age was 60.2 ± 14.3 years (range 27-85 years), and 61% were male. The vascular event was an ischemic stroke in 24 patients (55%) and a TIA in 20 patients (45%). The vascular event was caused by large artery atherosclerosis in 26 patients (59%), by cardioembolism in 10 patients (23%), by other determined aetiology, e.g. arterial dissection, in six patients (14%), and in two patients (5%) the cause was undetermined (according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification[21]). Microbleeds were found in 15 patients (34%) (6 (30%) of the TIA patients and 9 (38%) of the ischemic stroke patients). Of the patients with microbleeds, 7 (47%) showed 3 or more microbleeds. Patients with and without microbleeds did not differ in age, sex, type or aetiology of the vascular event, or vascular risk factor profile (table 1). In 36 patients the first MRI-scan was used for the analyses and in 8 patients the second MRI-scan. The presence of microbleeds was independent of which MRI scan (the first or the second) was analysed.

MRI findings are provided in table 2. Median time between the vascular event

Table 1. Patient characteristics (n = 44)

	No microbleeds n = 29	≥1 Microbleeds n = 15	≥3 Microbleeds n = 7
Sex (male)	18 (62%)	9 (60%)	3 (43%)
Age (years)	59.6 ± 14.8	61.3 ± 13.5	54.7 ± 12.2
Education level (1-7) (median (IQR))	5 (3; 6)	5 (4; 6)	5 (4; 6)
Clinical classification			
Ischemic stroke diagnosis	15 (52%)	9 (60%)	4 (57%)
TIA diagnosis	14 (48%)	6 (40%)	3 (43%)
Ischemic stroke/TIA mechanism [a]			
Atherothrombotic	18 (62%)	8 (53%)	2 (29%)
Cardioembolic	5 (17%)	5 (33%)	3 (43%)
Other determined aetiology	5 (17%)	1 (7%)	1 (14%)
Undetermined	1 (3%)	1 (7%)	1 (14%)
Systolic blood pressure (mm Hg)	147 ± 25	149 ± 27	133 ± 23
Hypertension	15 (52%)	10 (67%)	4 (57%)
Hypercholesterolemia	10 (34%)	4 (27%)	1 (14%)
Diabetes mellitus	6 (21%)	3 (20%)	2 (29%)
Current smoking	12 (41%)	5 (33%)	3 (43%)

IQR = interquartile range

Data are presented as mean ± standard deviations or n (%) unless otherwise specified

[a] According to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification[21]

and the MRI scan was 7 days (range 1-93 days). A total of 75 microbleeds were detected in 15 patients. They were equally found in lobar regions (n=38; 51%) and in deep or infratentorial regions (n=37; 49%). More patients showed strictly lobar microbleeds (n=10; 23%) than strictly infratentorial or deep microbleeds (n=2; 5%), p=0.095. Lacunar infarcts were found significantly more often in patients with than in patients without microbleeds (47% vs 7%, p<0.05). Other MRI markers (i.e. WMH scores, large vessel infarcts, atrophy) did not differ between patients with and without microbleeds (all p>0.05).

Median time between the vascular event and neuropsychological assessment was 50 days (range 4-210 days). Timing of neuropsychological assessment did not

Table 2. MRI findings

Time interval vascular event – MRI scan (days) (median (range))
Microbleeds
Total number of microbleeds (median (range))
Number of deep or infratentorial microbleeds (median (range))
Number of lobar microbleeds (median (range))
Strictly deep or infratentorial microbleeds
Strictly lobar microbleeds
Other MRI measures
Lacunar infarcts (present; median (range))
Large vessel infarct
No infarct
WMH load (0-24) (median (IQR)) [a]
Cortical atrophy (0-3) [b]
Subcortical atrophy (0-3) [c]

WMH = white matter hyperintensity

Data are presented as mean ± standard deviations or n (%) unless otherwise specified

[a] WMH were scored according to the Age-Related White Matter Changes (ARWMC) scale[20]

[b] Measured semi quantitatively with a scale (0-3) based on the width of the Sylvian and interhemispheric fissure

[c] Measured semi quantitatively with a scale (0-3) based on the size of the frontal horns and lateral ventricles

* significantly different from group without microbleeds

No microbleeds n = 29	≥1 Microbleeds n = 15	≥3 Microbleeds n = 7
6.5 (1-93)	6.5 (3-79)	6 (3-53)
-	2 (1-33)	4 (3-33)
-	0 (0-31)	0 (0-31)
-	2 (0-12)	3 (1-12)
-	2 (13%)	0
-	10 (67%)	4 (57%)
2 (7%); 0 (0-3)	4 (47%); 0 (0-2) *	3 (43%); 0 (0-2) *
17 (63%)	10 (67%)	5 (71%)
9 (33)	2 (13)	1 (14)
3 (1; 6)	4 (3; 5)	3 (2; 5)
1.3 ± 0.7	1.6 ± 0.7	1.7 ± 0.8
1.6 ± 0.8	1.7 ± 0.8	1.5 ± 0.9

differ between patients with and without microbleeds. Mean MMSE score was 28.5 ± 1.9 (range 22-30); 28.6 ± 2.2 for patients without and 28.4 ± 2.2 for patients with microbleeds (p=0.144). Patients with microbleeds performed slightly worse on all four cognitive domains than those without (age and sex adjusted mean differences in z-scores (95% CI) between patients with and without microbleeds for memory: -0.08 (-0.52; 0.36); for language: -0.14 (-0.60; 0.31); for executive functioning: -0.15 (-0.57; 0.27); for visuoconstruction and -perception: -0.16 (-0.67; 0.35); for composite cognition: -0.16 (-0.67; 0.36)), but differences were not statistically significant. Similar results were found in patients with multiple microbleeds (table 3).

In contrast to the findings for microbleeds, other markers of SVD on MRI were related to cognition. Atrophy was associated with lower composite cognition (standardized regression coefficient β (adjusted for age, sex, and additionally for educational level) for cortical atrophy: -0.39 (-0.69; -0.08), p=0.014; for subcortical atrophy: -0.57 (-0.91; -0.23), p=0.002) and also a higher total WMH score was related with lower composite cognition (-0.45 (-0.80; -0.10), p=0.013). The presence of lacunar or large vessel infarcts on MRI was not related to cognition.

Table 3. Cognitive performance

	No microbleeds n = 29	≥1 Microbleeds n = 15	≥3 Microbleeds n = 7
MMSE (0-30) (mean ± SD)	28.6 ± 2.2	28.4 ± 1.2	29 ± 1.0
Time interval event – NPA (median days (range))	45 (4-210)	51 (6-79)	53 (6-75)
Cognitive performance [a]			
Memory	0.03 ± 0.66	-0.06 ± 0.86	-0.04 ± 0.57
Language	0.08 ± 0.83	-0.11 ± 0.69	-0.11 ± 0.73
Executive functioning	0.05 ± 0.70	-0.11 ± 0.61	-0.02 ± 0.80
Visuoconstruction and –perception	0.06 ± 0.69	-0.09 ± 0.90	-0.28 ± 0.92
Composite score	0.06 ± 0.59	-0.09 ± 0.54	-0.11 ± 0.50

NPA = neuropsychological assessment

[a] Data are presented as mean standardized z-scores ± SD.

None of the variables differed statistically significant between the groups.

DISCUSSION

In patients with a TIA or ischemic stroke in the anterior circulation, we found no association between the presence of microbleeds at 7T MRI and cognitive performance in four cognitive domains.

To the best of our knowledge, this is the first study on microbleeds at ultra-high field strength MRI in patients who have suffered from a recent TIA or ischemic stroke. As the detectability of microbleeds increases with increasing field strength, this might influence associations with other clinical characteristics. We found a prevalence of microbleeds of 34%, which is indeed higher than in previously published studies that used regular field strength (1.0-3.0 T) MRI. In a meta-analyses including published studies from 1999-2004, a combined prevalence of 21.5% in patients with ischemic stroke was reported.[6] In subsequent studies, prevalences up to 32% are mentioned (e.g. [22–25]), comparable with our prevalence. The latter studies included patients with large vessel as well as lacunar ischemic stroke.[22,23,25] In our study the prevalence of microbleeds has probably been influenced by the selection of patients with large vessel infarcts, since microbleeds are more common in patients with a lacunar stroke than in patients with a large vessel stroke.[6] Furthermore, we

assessed relatively young patients and included also patients with a TIA, which could partly explain a relatively lower microbleed prevalence as well.[6]

Previous studies on the relationship between microbleeds and cognitive performance in patients with a TIA or stroke reported inconsistent results. In the general population, microbleeds have been related to cognitive impairment (mostly in the cognitive domains of executive functioning and information processing speed), although observed cognitive decrements in persons with microbleeds compared to persons without microbleeds were relatively small.[9,10] In patients with a TIA or ischemic stroke, however, these associations were less clear.[11-13,26-28] Some studies reported relationships between the presence of microbleeds and cognitive performance, but this was mostly restricted to decrements in executive functioning, and did not extend to other domains.[11-13,26] Moreover, associations with cognition were mainly observed for specific subgroups of patients with microbleeds, particularly those with lobar[26] or multiple[13] microbleeds. Other studies found no relation between microbleeds and cognitive performance, such as recently in patients with a lacunar stroke.[29] Moreover, we previously reported no association between microbleeds at regular field strength and global cognitive performance in 328 patients with a TIA or minor ischemic stroke.[28] In spite of the great variability in MRI techniques, investigated populations, and cognitive rating instruments used, these findings show that the relationship between microbleeds and cognitive dysfunction in patients with an ischemic stroke or TIA is at least not very strong. A possible explanation is that in these patient groups many persons will have SVD, including patients without microbleeds. In contrast, in the general population, the overall burden of vascular pathology will be lower, increasing the contrast between persons with and those without MRI markers of SVD, such as microbleeds.

Strengths of our study include the precise assessment of microbleeds with a dedicated protocol at 7T MRI, and the detailed neuropsychological examination. An important limitation is that we investigated a subgroup of patients with ischemic stroke or TIA, i.e. only patients with a TIA or ischemic stroke of the anterior circulation. Therefore, conclusions cannot simply be extrapolated to all patients with a TIA or ischemic stroke. Furthermore, the aetiology of these strokes was heterogeneous (table 1). This could have influenced our results, since different aetiologies may have different concomitant SVD and a differential impact on cognition. Lastly, some patients showed no infarct on brain MRI, while others did, and present ischemic brain lesions differed in size and localization, which will have different effects on cognition as well. Further limitations are the variation in timing of the neuropsychological assessment, and the modest sample size. The stringent

contra-indications for 7T MRI and the poor clinical condition of some patients in the first week after their stroke, prevented a number of inclusions, which might have caused selection bias. However, the fact that brain atrophy and WMH scores were related to cognitive performance in our cohort is well in line with previous studies showing similar associations in patients with ischemic stroke.[30,31] This indicates that low statistical power cannot completely explain the absence of a relationship between microbleeds and cognition. Finally, due to the limited number of patients with multiple microbleeds, we could not reliably investigate whether high microbleed counts were related to cognitive impairment, as has been suggested before.[13]

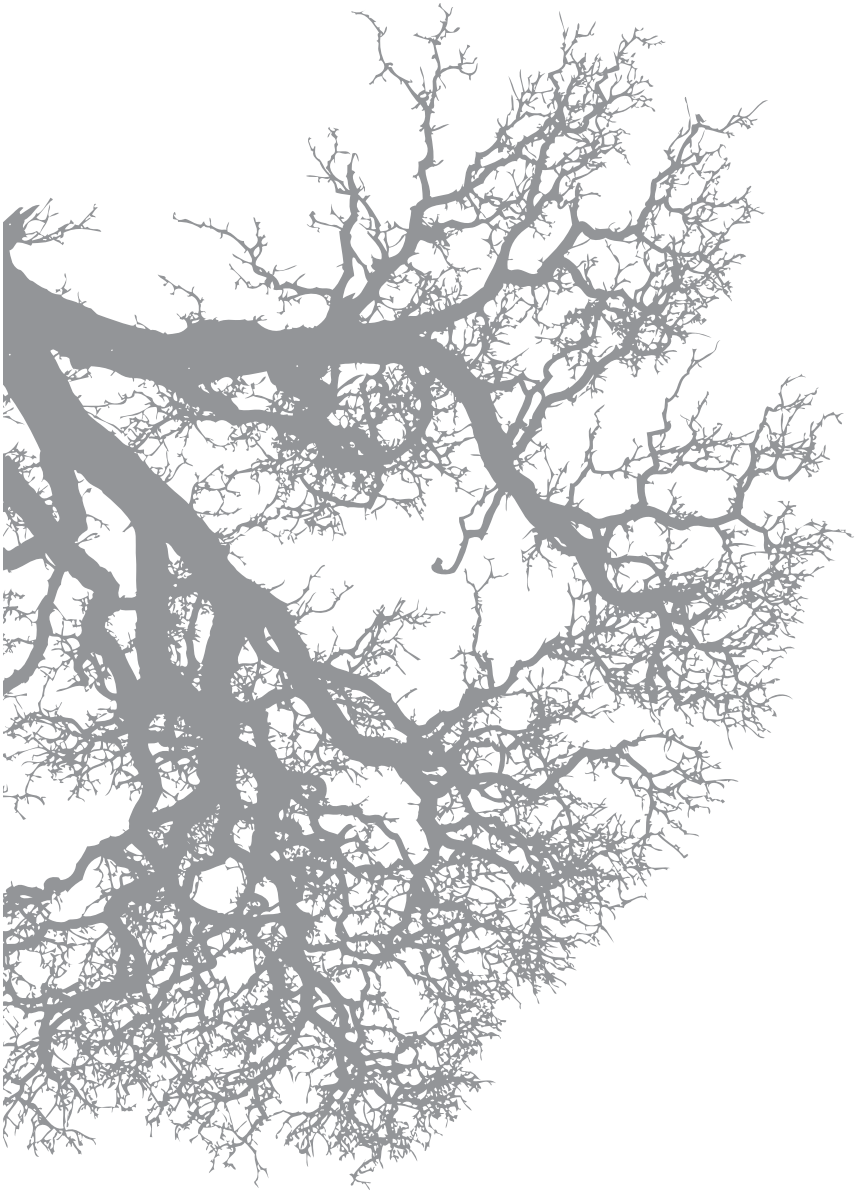
In conclusion, also when microbleeds are assessed at 7T MRI, they did not contribute to cognitive impairment in patients with large vessel ischemic stroke or TIA.

REFERENCES

1. Ihle-Hansen H, Thommessen B, Wyller TB, et al. Incidence and subtypes of MCI and dementia 1 year after first-ever stroke in patients without pre-existing cognitive impairment. *Dement Geriatr Cogn Disord*. 2011;32:401–407.
2. Savva GM, Stephan BC. Epidemiological studies of the effect of stroke on incident dementia: a systematic review. *Stroke* 2010;41:e41–e46.
3. Mok VCT, Wong A, Lam WWM, Fan YH, Tang WK, Kwok T, Hui ACF, Wong KS. Cognitive impairment and functional outcome after stroke associated with small vessel disease. *J Neurol Neurosurg Psychiatry* 2004;75:560–566.
4. Pantoni,L. Cerebral small vessel disease:from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol*. 2010;9:689–701.
5. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, Barkhof F, Benavente OR, Black SE, Brayne C, Breteler M, Chabriat H, Decarli C, de Leeuw FE, Doubal F, Duering M, Fox NC, Greenberg S, Hachinski V, Kilimann I, Mok V, Van Oostenbrugge R, Pantoni L, Speck O, Stephan BC, Teipel S, Viswanathan A, Werring D, Chen C, Smith C, van Buchem M, Norrving B, Gorelick PB, Dichgans M; STandards for ReportIng Vascular changes on nEuroimaging (STRIVE v1). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* 2013;12:822-838.
6. Cordonnier C, Al-Shahi Salman R, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain* 2007;130:1988-2003.
7. Poels MMF, Vernooij MW, Ikram MA, Hofman A, Krestin GP, van der Lugt A, Breteler MMB. Prevalence and Risk Facotrs of Cerebral Microbleeds: An Update of the Rotterdam Scan Study. *Stroke* 2010;41:S103-S106.
8. Cordonnier C, van der Flier WM. Brain microbleeds and Alzheimer's disease: innocent observation or key player? *Brain* 2011;134:335-344.
9. 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. *Neurology* 2012;78:326-333.
10. Qiu C, Cotch MF, Sigurdsson S, Jonsson PV, Jonsdottir MK, Sveinbjrnsdottir S, Eiriksdottir G, Klein R, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. *Neurology* 2010;75:2221-2228.
11. Gregoire SM, Scheffler G, Jager 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* 2013;44:1267-1272.
12. Werring DJ, Frazer DW, Coward LJ, Losseff NA, Watt H, Cipolotti L, Brown MM, Jager HR. Cognitive dysfunction in patients with cerebral microbleeds on T2*-weighted gradient-echo MRI. *Brain* 2004;127:2265-2275.
13. Patel B, Lawrence AJ, Chung AW, Rich P, Mackinnon AD, Morris RG, Barrick TR, Markus HS. Cerebral microbleeds and cognition in patients with symptomatic small vessel disease. *Stroke* 2013;44:356-361.
14. Conijn MM, Geerlings MI, Biessels GJ, Takahara T, Witkamp TD, Zwanenburg JJ, Luijten PR, Hendrikse J. Cerebral microbleeds on MR imaging: comparison between 1.5 and 7T. *AJNR Am J Neuroradiol* 2011;32:1043-1049.
15. 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 Neurol* 2009;8:165-174.
16. Van der Kolk AG, Zwanenburg JJ, Brundel M, Biessels GJ, Visser F, Luijten PR, Hendrikse J. Intracranial vessel wall imaging at 7.0-T MRI. *Stroke* 2011;42:2478-84.
17. Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet* 1991;337:1521-1526.
18. Kuijf HJ, de Bresser J, Geerlings MI, Conijn MM, Viergever MA, Biessels GJ, Vincken KL. Efficient detection of cerebral microbleeds on 7.0 T MR images using the radial symmetry transform. *Neuroimage* 2012;59:2266-2273.
19. Kuijf HJ, Brundel M, de Bresser J, van Veluw SJ, Heringa SM, Viergever MA, Biessels GJ, Vincken KL. Semi-Automated Detection of Cerebral Microbleeds on 3.0 T MR Images. *PLoS One*. 2013;8:e66610.
20. 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, European Task Force on Age-Related White Matter Changes. A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke* 2001;32:1318-1322.
21. Adams HP Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE 3rd. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993;24:35-41.
22. Cho AH, Lee SB, Shon YM, Yang DW, Kim BS. Impaired kidney function and cerebral microbleeds in patients with acute ischemic stroke. *Neurology* 2009;73:1645-1648.
23. Seo WK, Lee JM, Park MH, Park KW, Lee DH. Cerebral microbleeds are independently associated with arterial stiffness in stroke patients. *Cerebrovasc Dis* 2008;26:618-623.
24. Ovbiagele B, Liebeskind DS, Pineda S, Saver JL. Strong independent correlation of proteinuria with cerebral microbleeds in patients with stroke and transient ischemic attack. *Arch Neurol* 2010;67:45-50.

25. Jeon SB, Kwon SU, Cho AH, Yun SC, Kim JS, Kang DW. Rapid appearance of new cerebral microbleeds after acute ischemic stroke. *Neurology*. 2009;73:1638-44.
26. Gregoire SM, Smith K, Jager HR, Benjamin M, Kallis C, Brown MM, Cipolotti L, Werring DJ. Cerebral microbleeds and long-term cogntive outcome: longitudinal cohort study of stroke clinic patients. *Cerebrovasc Dis* 2012;33:430-435.
27. Zhang M, Chen M, Wang Q, Yun W, Zhang Z, Yin Q, Huang Q, Zhu W. Relationship between cerebral microbleeds and cognitive function in lacunar infarct. *J Int Med Res* 2013;41:347-355.
28. Brundel M, Kwa VI, Bouvy WH, Algra A, Kappelle LJ, Biessels GJ. Cerebral Microbleeds Are Not Associated with Long-Term Cognitive Outcome in Patients with Transient Ischemic Attack or Minor Stroke. *Cerebrovasc Dis* 2014;37:195-202.
29. Lawrence AJ, Patel B, Morris RG, MacKinnon AD, Rich PM, Barick TR, Markus HS. Mechanisms of cognitive impairment in cerebral small vessel disease: multimodal MRI results from the St George's Cognition and Neuroimaging in Stroke (SCANS) Study. *PloS One* 2013;8:61014.
30. Tang WK, Chan SS, Chiu HF, Ungvari GS, Wong KS, Kwok TC, Mok V, Wong KT, Richards PS, Ahuja AT. Frequency and determinants of poststroke dementia in Chinese. *Stroke* 2004;35:930–935.
31. Stebbins GT, Nyenhuis DL, Wang C, Cox JL, Freels S, Bangen K, de Toledo-Morrell L, Sripathirathan K, Moseley M, Turner DA, Gabrieli JD, Gorelick PB. Gray matter atrophy in patients with ischemic stroke with cognitive impairment. *Stroke* 2008;39:785-793.



11 GENERAL DISCUSSION

This thesis focuses on brain imaging markers of vascular cognitive impairment (VCI). The term VCI refers to the entire spectrum of cognitive disorders associated with and presumed to be caused by any form of cerebrovascular disease.
The main aims of this thesis were 1) to assess different brain imaging markers of type 2 diabetes mellitus, as a vascular risk factor for cognitive decline; and 2) to explore cerebral microvascular lesions (microinfarcts and microbleeds) on MRI as an etiological and prognostic marker of VCI.

The main findings of this thesis and implications for clinical care and future research are discussed in this chapter. Part II of this thesis, focusing on microvascular lesions, will be discussed first, as it also has implications for part I.

MICROVASCULAR LESIONS

Cerebral small vessel disease (SVD), an important underlying pathophysiological process of VCI, can be studied with MRI, although the small vessels themselves cannot directly be visualized with conventional MRI techniques. Instead, the lesions that develop in the brain tissue as a consequence of abnormalities in the small vessels are used as markers to detect SVD. Traditionally these include white matter hyperintensities (WMH) and lacunar infarcts. Over the last decades, other MRI markers of SVD have been introduced, such as microbleeds. Furthermore, from autopsy studies it became clear that microinfarcts are another feature of SVD that are likely to play an important role in cognitive impairment and dementia.

Microinfarcts

Detection of microinfarcts at 7T MRI

In autopsy studies, microinfarcts are defined as ischemic lesions "not visible with the naked eye" or "only visible upon light microscopy". Because of their limited size, microinfarcts could until recently not be detected on conventional MRI. However, the past decade, ultra-high field strength MRI has been introduced, which offers higher spatial resolution. When I started my research project in 2009, we wanted to explore the possibility to visualize microinfarcts in vivo, with the new 7T MRI scanner, in order to investigate the meaning of these lesions in VCI.

The difficulty at that moment was that we did not know what microinfarcts on MRI would look like. Therefore, we systematically reviewed available neuropathological studies on cerebral microinfarcts, as a frame of reference for our MRI studies (**chapter 6**). These revealed that microinfarcts are relatively common

at older age (24%). Microinfarcts are even more common among older individuals with vascular dementia (62%), Alzheimer's Disease (AD) (43%) and mixed forms of dementia (33%). They are described as minute foci with neuronal loss, gliosis, pallor or more cystic lesions. Microinfarcts are found in all brain regions, possibly more so in the cerebral cortex, particularly in watershed areas. Reported sizes vary from 50 μm to a few mm. Microinfarcts have been associated with cognitive performance or a clinical diagnosis of dementia before death and with other forms of SVD-related brain damage.

Based on these histopathological characteristics of microinfarcts, i.e. sharply delineated microscopic ischemic lesions, often associated with gliosis and cavitation, and on knowledge of the MR appearance of other ischemic lesions, we developed a scan protocol, to visualize cortical microinfarcts in vivo. We hypothesized that microinfarcts would appear as small hyperintense circumscript (with or without a hypointense center) lesions on FLAIR images, hyperintense on T_2-weighted images, and hypointense or isointense on T_1-weighted images. We focused on microinfarcts in the cerebral cortex, because we expected that in the subcortical gray and white matter it would be more difficult to differentiate microinfarcts from other focal lesions, e.g. WMH and perivascular spaces. After intensive evaluation of the 7T MRI scans, we came across lesions with features that matched our assumptions. Next, we performed MRI scans of post-mortem brain material, which contained the same lesions. These lesions were examined histologically, and we could indeed confirm that they were microinfarcts.[1] Based on these findings, we defined criteria for possible microinfarcts on in vivo 7T MRI (as described above; furthermore microinfarcts had to be detectable on sagittal, coronal, and transversal views of the brain and restricted to the cortex).[1]

The above mentioned MR sequences were already incorporated in research scan protocols of different clinical studies. Because we were among the first in the world scanning large numbers of patients on 7T MRI, we were faced a number of practical issues. Because of the limited experience with 7T MRI - at least initially - very stringent contra-indications with respect to metal objects were applied, much more stringent than for regular clinical MR scanners. These rules, although appropriate from a safety perspective, prevented inclusion of a substantial number of people, especially elderly. Fortunately, over the past years our knowledge on safety aspects of the scanner have increased considerably, leading to a marked shortening of the list of contra-indications for 7T MR scanning. This important work should be continued, since it is essential for patient safety, but will also reduce selection bias when contraindications that prove to be unnecessary from a safety perspective can be dropped. Furthermore, experimental scan protocols at 7T MRI are often long,

which can be a problem in older or diseased persons. The focus should therefore be on dedicated scan protocols to detect microvascular lesions accurately, but with the shortest scan times to minimize scan artefacts. Finally, the availability of 7T scanners is limited. The accessibility of 7T scanners should be increased, to make future research and also clinical implementation more feasible. On the other hand, optimization of detection of microinfarcts at lower field strength (3T), as has been demonstrated recently,[1,2] will also increase the dissemination of this marker in research and possibly clinic.

Rating of microinfarcts

After we succeeded in visualizing cortical microinfarcts at 7T MRI, we experienced a number of difficulties in rating these lesions. Inter-rater agreement is moderate (intraclass correlation coefficient on the order of 0.40). Defining lesions as microinfarcts is rater dependent and remains a challenge, despite stringent criteria. Because lesions are small, they are susceptible for movement artefacts. Secondly, with our MRI scan protocol, we cannot detect lesions of ≤1 mm in size, in contrast to neuropathology studies where lesions of 50 μm in size can be detected. It is therefore uncertain whether MRI detected lesions are a good representation of the complete lesion load in the brain. Finally, rating microinfarcts at MRI is time consuming. The use of semi-automated detection tools offers practical benefits and can increase both detection of microinfarcts and inter-rater agreement. This has also been shown for microbleeds,[3] and have already been used in chapters 5, 7, and 10 of this thesis.

Microinfarcts in different study populations

Thus, it became possible to study cortical microinfarcts in vivo at 7T MRI in different populations. We showed that in nondemented persons, microinfarcts are common and already detectable at middle-age (**chapter 7**). The overall occurrence (38%) was higher than we found in our review of autopsy studies (24%), while the persons we investigated were younger than in these neuropathological studies.

Microinfarcts on MRI were unrelated to age, vascular risk factors or other markers of SVD (**chapter 7**). We also found no increased occurrence of microinfarcts in patients with T2DM compared to controls. Furthermore, the presence of microinfarcts was not associated with cognitive performance in persons aged 65-80 with or without T2DM (**chapter 5**).

There thus appears to be a discrepancy between the links between microinfarcts and age, other imaging markers of SVD, and cognition in our in vivo studies and in

previous neuropathological studies. This might be caused by the fact that the latter investigated much older persons (mean age at death in most studies above 80), with a high probability of ageing related abnormalities and end-stage disease, including both Alzheimer's pathology and cerebrovascular disease, also in individuals without clinical manifestations of dementia prior to death. Along these lines, two recent in vivo 7T MRI studies showed that microinfarcts were not more prevalent in patients with early AD,[4] but were indeed more prevalent in patients with more advanced AD[5] than in controls. Moreover, the presence of microinfarcts was only related to cognitive performance in patients with more advanced AD.[5] It seems to be a paradox that on the one hand microinfarcts are frequently seen, also in younger persons without cerebrovascular disease or cognitive disturbances, whereas on the other hand they are associated with dementia and cerebrovascular disease. One explanation could be that at the population level, microinfarcts are in most cases not a manifestation of disease, but rather a marker for future risk of stroke or dementia. In other words, they could be a marker of other pathophysiological processes which causally lead to cognitive decline or stroke. Another explanation could be that they lead to dementia through synergistic or additive effects together with other disease processes. Hence, the relation with dementia would only become evident when these other processes also occur. Finally, the relation between microinfarcts and disease could be not-linear, i.e. persons with a mild lesion load will not develop cognitive symptoms, but patients with a higher lesion load do.

Microbleeds

7T MRI is also very sensitive for detection of microbleeds. Microbleeds consist of hemosiderin deposits that are paramagnetic, which induces a susceptibility effect: it leads to a fast decay of the local T_2^*-weighted MRI signal, visible as a signal void. With higher field strength, the susceptibility effect increases, and increased signal-to-noise ratio can be used for higher spatial resolution. We used this higher sensitivity for detection of microbleeds to assess the association with ageing and other markers of SVD (**chapter 7**), to assess the occurrence in patients with T2DM, as a risk factor for cerebrovascular disease and dementia (**chapter 5**), and to assess the occurrence and relationship with cognitive performance in patients with AD (**chapter 8**) and ischemic stroke (**chapter 10**).

We showed that prevalences of microbleeds at 7T MRI were indeed higher than in previous studies at regular field strength. Reported microbleed prevalences at conventional field strength in two systematic reviews were 5% in the general population, 23% in patients with AD, and 21.5% in patients with ischemic stroke,[6,7]

while we found microbleeds in 38% of the healthy adults (**chapter 7**), 78% of patients with early AD (**chapter 8**), and 34% of patients with a TIA or ischemic stroke (**chapter 10**). However, with this sensitive imaging technique, not all of our findings on the clinical and functional correlates of microbleeds were in accordance with previous studies at regular field strength.

In **chapter 7**, we investigated "healthy persons" (i.e. people without major neurological conditions). We demonstrated that in these persons microbleeds are related to age, which is well in line with previous studies in the general population,[8-10] although the reported odds ratios were higher than in our study. This difference could be the result of the higher sensitivity of our imaging method resulting in the detection of smaller lesions, also in younger persons.

Previous studies at regular field strength have linked the presence of microbleeds to vascular risk factors and other MR markers of SVD, but not invariably. For example, a meta-analysis that included the earliest studies, showed that microbleeds appear to be associated with hypertension and diabetes mellitus in the general population,[6] while in more recent large population-based cohorts, no or weak relationships were found, in line with our study.[8,10] Furthermore, some studies reported an association between microbleeds and lacunar infarcts[11,12] or higher WMH scores,[11] but others found only a relationship with deep or infratentorial microbleeds[8] or no relationship at all.[9] In our sample we did not find a relationship between microbleeds and vascular risk factors or other MR markers of SVD.

In **chapter 5**, we investigated patients with T2DM. We did not find an increased occurrence of microbleeds compared to controls, in line with two recent studies at regular field strength.[13,14] Of note, we assessed a relatively well-controlled patient group. Further studies should assess whether they do occur more often in patients who are less well controlled or have a high burden of microvascular complications elsewhere in the body.

We also did not find a relationship between microbleeds and cognitive performance in the patients with T2DM or the controls. In previous large population-based cohorts, not specifically addressing T2DM, only weak associations between microbleeds and modest cognitive deficits, mostly in the cognitive domains of executive functioning and information processing speed, have been reported,[15,16] for example 0.4 MMSE points, for people with ≥5 microbleeds relative to those without.[16] Despite the high sensitivity of ultra-high field strength MRI, it does not add greater clarity to the relationship between microbleeds and cognition. In fact, this association seems to be rather weak in older persons without dementia.

In **chapter 8**, we showed that microbleeds measured at 7T MRI were more prevalent in patients with early AD (78%) than in controls (44%). Cerebral amyloid angiopathy (CAA) is presumed to be the main neuropathological correlate of microbleeds in AD. The fact that microbleeds were present in the majority of patients with early AD, fits with fits with the high prevalence of CAA in AD in neuropathological studies.[17] Microbleeds were not related to MMSE score within the patient group either. Previous studies in patients with AD also reported no association between microbleeds and cognitive performance.[18-22] Only one study reported microbleeds to be related to cognition in patients with AD, but this concerned patients with eight or more microbleeds.[23]

Finally, in **chapter 10**, we investigated microbleeds on 7T MRI in patients with a TIA or ischemic stroke. Also in these patients, microbleeds were not related to cognitive performance in four cognitive domains. Previous studies at regular field strength investigating a relationship between microbleeds and cognition in patients with an ischemic stroke or TIA, reported inconsistent results. Reported associations were rather weak, and restricted to for example specific cognitive domains[24-27] or specific subgroups of patients with lobar[24] or multiple[27] microbleeds. Thus, the relationship between microbleeds and cognitive performance in patients with a TIA or ischemic stroke seems to be at least not very strong, also when investigated at ultra-high field strength MRI. A possible explanation is that in these patient groups many persons will have SVD, including patients without microbleeds. In contrast, in the general population, the overall burden of vascular pathology will be lower, increasing the contrast between persons with and those without MRI markers of SVD, such as microbleeds.

Nowadays, 1.5T MRI is much more available than 7T MRI and still widely used in daily practice. Therefore, we also investigated the relationship between microbleeds at regular field strength and cognition in 328 patients with a TIA or ischemic stroke, representative of a regular vascular outpatient clinic population (**chapter 9**). As to be expected, we found a lower microbleed prevalence (11.6%) than at 7T MRI, but the presence of microbleeds in this cohort was also not related to global cognitive performance. This is well in line with our results of chapter 10, and with previous studies as described above.

To summarize, microbleeds on 7T MRI are quite common, also in apparently healthy older individuals. They are not strongly related to other markers of SVD.

In healthy individuals, microbleeds are not clearly associated with cognition, except for subgroups of persons with a high lesion load. Microbleeds are more common in patients with stroke or dementia than in healthy individuals, but not clearly related to cognitive performance in these patients groups. It appears that at the population-level, similarly to microinfarcts, microbleeds could be a marker of future risk for stroke and cognitive decline. At the same time, in a subgroup, they could be an early manifestation of a pathophysiological process, e.g. amyloid angiopathy in the context of AD. In patients with stroke or dementia, the overall burden of other vascular and neurodegenerative pathologies will be higher, which may mask the relatively subtle relation between microbleeds and cognition.

Detection of microvascular lesions at 7T MRI has several implications for future research, as described above. For clinical care, however, implications are at present less obvious. Although microbleeds are increasingly recognized as an imaging marker for SVD, research on microinfarcts in vivo has only recently been started, and there is still uncertainty on the clinical relevance of these lesions detected with MRI. Currently, diagnostic and prognostic meaning of both microbleeds and microinfarcts are unclear. Although an increasing number of papers point to the potential clinical relevance of microbleeds, this is still mostly based on expert opinion. Microbleeds have been suggested to increase the risk of future cerebrovascular accidents in patients with a past stroke.[6,28] The question has been raised if presence of microbleeds should influence the prescription of antithrombotic agents,[29] but it is still unknown if these agents increase the risk of future hemorrhages in patients with microbleeds.[28,30] The challenge for future research will be to determine which imaging markers – or more likely: which combination of imaging markers – have diagnostic and prognostic value for the individual patient.

BRAIN IMAGING MARKERS IN TYPE 2 DIABETES

Type 2 diabetes mellitus (T2DM) predisposes to macro- and microvascular disease, both in the brain and elsewhere in the body,[31] and is associated with cognitive dysfunction. These cognitive changes are attributable to structural abnormalities in the brain, which can be visualized with MRI. Brain MRI markers may therefore provide important clues for underlying mechanisms. **Chapter 2** presents a recent overview of brain imaging in T2DM. The main conclusion is that T2DM is associated with various forms of brain damage, mildly distinct from changes occurring in normal

aging. This heterogeneity is also reflected in the findings of chapters 3, 4 and 5.

Microvascular lesions, as a marker of SVD in T2DM (**chapter 5**), were already addressed in the previous section of this general discussion.

In **chapter 3**, we investigated cerebral cortical atrophy in patients with T2DM. Atrophy is an important marker of T2DM-related brain damage: available literature consistently shows that T2DM is associated with a modest degree of global brain atrophy – the equivalent of 3-5 years of normal brain aging – and a slightly increased atrophy rate over time, compared with persons without T2DM. The question remains if specific brain regions are more vulnerable to T2DM-related atrophy. In our sample, we found that T2DM was indeed associated with global cortical atrophy, and specifically with cortical atrophy of the medial temporal lobe. Others observed recently that T2DM-related gray matter loss was distributed mainly in medial temporal, anterior cingulate, and medial frontal lobes.[32] In previous studies, lower hippocampal volumes have also been reported in patients with T2DM.[33] However, when hippocampal volumes are measured and expressed as percentage of total brain volume, hippocampal atrophy is not out of proportion to global brain atrophy in T2DM.[34] Other parts of the medial temporal lobe appear to give rise to observed atrophy in this brain region. The pattern of brain atrophy in patients with T2DM may provide clues toward underlying mechanisms. T2DM has been associated with different pathophysiological processes involved in both vascular (e.g. endothelial dysfunction, pro-inflammatory and prothrombotic factors[35]) and neurodegenerative pathways (e.g. alterations in the phosphorylation of tau[36] and decreased breakdown of amyloid[37,38]). Future studies should link regional brain volumes to specific risk factors or markers for etiological processes (e.g. markers for perfusion, inflammation, or accumulation of amyloid).

Some previous studies showed diminished cerebral blood flow (CBF) or decreased cerebrovascular reactivity (CVR) in patients with T2DM compared to controls.[39,40] Cerebral haemodynamics might mediate the relationship between T2DM and brain abnormalities and cognitive dysfunction. We therefore investigated CBF and CVR in a longitudinal study on nondemented patients with T2DM (**chapter 4**). We found that CBF, but not CVR, was associated with baseline cognitive performance and total brain volume. Neither CBF nor CVR at baseline predicted change in cognition or in total brain volume after four years. These results emphasize the importance of longitudinal studies to investigate etiological processes. Our study does not support a causal role for disturbed haemodynamics in cognitive dysfunction and atrophy. Reverse causality might play a role. In other words, the demand for CBF may be relatively lower in abnormal atrophic brain tissue, thus giving rise to the observed

associations in the cross-sectional studies. This can also be concluded from previous cross-sectional studies, which did not correct CBF or CVR for brain atrophy, and decreased haemodynamics might therefore well be considered a consequence rather than a cause of cerebral atrophy. The techniques we used to measure CBF and CVR are non-invasive, fast and accurate. On the other hand, it only measures the total amount of blood flowing to the brain and the blood flow velocity in large cerebral arteries, while regional differences are also interestingly, as discussed in the light of brain atrophy. With other techniques, such as arterial spin labeling (ASL) or single-photon emission computed tomography (SPECT), regional differences can be measured and linked to other (regional) brain imaging markers.

Thus, brain damage in patients with T2DM should be considered as a combination of different processes, related to macro- and microvascular disease, both global and regional, and the increased risk of dementia in T2DM should be considered also in interaction with other pathologies (e.g. AD pathology). Future studies should focus on combining different imaging markers that add to the increased risk of dementia in T2DM in one prediction model.

Observed brain abnormalities in patients with T2DM are proven at the population-level. However, for the individual T2DM patient, brain changes are difficult to interpret, since they are generally subtle and there is no one-on-one relationship between MRI findings and cognitive performance. Currently, diagnostic and prognostic values of individual MRI markers are insufficient with regard to T2DM-related cognitive decline for application in clinical practice.

At present, no proven treatment is available to prevent or slow down cognitive decline in patients with T2DM. A few studies focused on the effects of intensive glycemic intervention. Unfortunately, no consistent evidence for benefit of glucose lowering agents with regard to cognition has been reported yet (e.g. the ACCORD-MIND study[41]). Therefore, the role of MRI in the diagnostic evaluation of a T2DM patient with cognitive complaints should be identical to that in every other person with cognitive complaints.

REFERENCES

1. Van Veluw SJ, Zwanenburg JJM, Engelen-Lee JY, Spliet WGM, Hendrikse J, Luijten PR, Biessels GJ. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. *J Cereb Blood Flow Metab* 2013;33:322–329.
2. Ii Y, Maeda M, Kida H, Matsuo K, Shindo A, Taniguchi A, Tomimoto H. In vivo detection of cortical microinfarcts on ultrahigh-field MRI. *J Neuroimaging*. 2013;23:28-32.
3. Kuijf HJ, de Bresser J, Geerlings MI, Conijn MM, Viergever MA, Biessels GJ, Vincken KL. Efficient detection of cerebral microbleeds on 7.0 T MR images using the radial symmetry transform. *Neuroimage* 2012;59:2266-2273.
4. Van Veluw SJ, Heringa SM, Kuijf HJ, Koek HL, Luijten PR, Biessels GJ. Cerebral cortical microinfarcts at 7Tesla MRI in patients with Early Alzheimer's Disease. *J Alzheimers Dis* 2014;39:163-167.
5. Van Rooden S, Goos JD, 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* 2014;270:205-211.
6. Cordonnier C, Al-Shahi Salman R, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for study design and reporting. *Brain* 2007;130:1988-2003.
7. Cordonnier C, van der Flier WM. Brain microbleeds and Alzheimer's disease: innocent observation or key player? *Brain* 2011;134:335-344.
8. Poels MM, Vernooij MW, Ikram MA, Hofman A, Krestin GP, van der Lugt A, Breteler MM. Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam scan study. *Stroke* 2010;41:S103-106.
9. 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;35:1831-1835.
10. Sveinbjornsdottir S, Sigurdsson S, Aspelund T, Kjartansson O, Eiriksdottir G, Valtysdottir B, Lopez OL, van Buchem MA, Jonsson PV, Gudnason V, Launer LJ. Cerebral microbleeds in the population based AGES-Reykjavik study: prevalence and location. *J Neurol Neurosurg Psychiatry* 2008;79:1002-1006.
11. Roob G, Schmidt R, Kapeller P, Lechner A, Hartung HP, Fazekas F. MRI evidence of past cerebral microbleeds in a healthy elderly population. *Neurology* 1999;52:991-994.
12. Horita Y, Imaizumi T, Niwa J, Yoshikawa J, Miyata K, Makabe T, Moriyama R, Kurokawa K, Mikami M, Nakamura M. [Analysis of dot-like hemosiderin spots using brain dock system]. *No Shinkei Geka*. 2003;31:263-267.
13. Moran C, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Munch G, Wood AG,

Forbes J, Greenaway TM, Pearson S, Srikanth V. Brain Atrophy in Type 2 Diabetes: Regional distribution and influence on cognition. *Diabetes Care* 2013;36:4036-4042.

14. Qiu C, Sigurdsson S, Zhang Q, Jonsdottir MK, Kjartansson O, Eiriksdottir G, Garcia ME, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Diabetes, markers of brain pathology, and cognitive cognition: The AGES-Reykjavik study. *Ann Neurol* 2014;75:138-146.
15. Qiu C, Cotch MF, Sigurdsson S, Jonsson PV, Jonsdottir MK, Sveinbjrnsdottir S, Eiriksdottir G, Klein R, Harris TB, van Buchem MA, Gudnason V, Launer LJ. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. *Neurology* 2010;75:2221-2228.
16. Poels MM, Ikram MA, van der Lugt A, Hofman A, Niessen WJ, Krestin GP, Breteler MM, Vernooij MW. Cerebral microbleeds are associated with worse cognitive function: The Rotterdam Scan Study. *Neurology* 2012;78:326-333.
17. Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. *J Neural Transm* 2002;109:813-836.
18. Pettersen JA, Sathiayamoorthy G, Gao FQ, 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. *Arch Neurol* 2008;65:790-795.
19. Hanyu H, Tanaka Y, Shimizu S, Takasaki M, Abe K. Cerebral microbleeds in Alzheimer's disease. *J Neurol* 2003;250:1496-1497.
20. 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. *Ann N Y Acad Sci* 2002;977:169-172.
21. 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. *Dement Geriatr Cogn Disord* 2006;22:8-14.
22. Van der Vlies AE, Goos JD, Barkhof F, Scheltens P, van der Flier WM. Microbleeds do not affect rate of cognitive decline in Alzheimer disease. *Neurology* 2012;79:763-769.
23. Goos JD, 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;40:3455-3460.
24. Gregoire SM, Smith K, Jager HR, Benjamin M, Kallis C, Brown MM, Cipolotti L, Werring DJ. Cerebral microbleeds and long-term cogntive outcome: longitudinal cohort study of stroke clinic patients. *Cerebrovasc Dis* 2012;33:430-435.
25. Gregoire SM, Scheffler G, Jager 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* 2013;44:1267-1272.

26. Werring DJ, Frazer DW, Coward LJ, Losseff NA, Watt H, Cipolotti L, Brown MM, Jager HR. Cognitive dysfunction in patients with cerebral microbleeds on T2*-weighted gradient-echo MRI. *Brain* 2004;127:2265-2275.
27. Patel B, Lawrence AJ, Chung AW, Rich P, Mackinnon AD, Morris RG, Barrick TR, Markus HS. Cerebral microbleeds and cognition in patients with symptomatic small vessel disease. *Stroke* 2013;44:356-361.
28. Kwa VI, Algra A, Brundel M, Bouvy W, Kappelle LJ. Microbleeds as a predictor of intracerebral haemorrhage and ischaemic stroke after a TIA or minor ischaemic stroke: a cohort study. *BMJ Open* 2013;3:e002575.
29. 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 Neurol* 2009;8:165-174.
30. Vernooij MW, Haag MD, van der LA, Hofman A, Krestin GP, Stricker BH, Breteler MM. Use of Antithrombotic Drugs and the Presence of Cerebral Microbleeds: The Rotterdam Scan Study. *Arch Neurol* 2009;66:714-720.
31. Luitse MJ, Biessels GJ, Rutten GE, Kappelle LJ. Diabetes, hyperglycaemia, and acute ischaemic stroke. *Lancet Neurol* 2012;11:261-271.
32. Moran C, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Munch G, Wood AG, Forbes J, Gerenaway TM, Pearson S, Srikanth V. Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. *Diabetes Care* 2013;36:4036-4042.
33. Gold SM, Dziobek I, Sweat V, Tirsi A, Rogers K, Bruehl H, Tsui W, Richardson S, Javier E, Convit A. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. *Diabetologia* 2007;50:711-719.
34. Wisse LM, de Bresser J, Geerlings MI, Reijmer YD, Portegies MLP, Brundel M, Kappelle LJ, van der Graaf Y, Biessels GJ, on behalf of the Utrecht Diabetic Encephalopathy Study Group and the SMART-MR study group. Global brain atrophy but not hippocampal atrophy is related to type 2 diabetes. In revision.
35. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. *Lancet Neurol* 2006;5:64-74.
36. Hong M, Lee VM. Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons. *J Biol Chem* 1997;1:19547-19553.
37. Edbauer D, Willem M, Lammich S, Steiner H, Haass C. Insulin-degrading enzyme rapidly removes the beta-amyloid precursor protein intracellular domain (AICD). *J Biol Chem* 2002;19:13389-13393.
38. Qiu WQ, Walsh DM, Ye Z, Vekrellis K, Zhang J, Podlisny MB, Rosner MR, Safavi

A, Hersh LB, Selkoe D.J. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J Biol Chem* 1998;4:32730-32738.

39. Last D, Alsop DC, Abduljalil AM, Marquis RP, de Bazelaire C, Hu K, Cvallerano J, Novak V. Global and regional effects of type 2 diabetes on brain tissue volumes and cerebral vasoreactivity. *Diabetes Care* 2007;30:1193-1199.
40. Novak V, Last D, Alsop DC, Abduljalil AM, Hu K, Lepicovsky L, Cavallerano J, Lipsitz LA. Cerebral blood flow velocity and periventricular white matter hyperintensities in type 2 diabetes. *Diabetes Care* 2006;29:529-534.
41. Launer LJ, Miller ME, Williamson JD et al. Effects of intensive glucose lowering on brain structure and function in people with type 2 diabetes (ACCORD MIND): a randomised open-label substudy. *Lancet Neurol* 2011;10: 969-977.

12

SUMMARY

SUMMARY

SUMMARY

In our aging society, cognitive impairment and dementia are a major public health problem. The most common causes of dementia are Alzheimer's Disease (AD) and Vascular Dementia (VaD), historically considered as two separate entities. The past decades, however, this tight categorical view is being re-evaluated and the role of vascular brain pathology in cognitive decline is increasingly recognized, also in patients who are diagnosed with AD. The term Vascular Cognitive Impairment (VCI) has been introduced, to refer to the entire spectrum of cognitive disorders associated with and presumed to be caused by all forms of cerebrovascular disease. It thus refers to the contribution of vascular brain damage across the whole spectrum of AD to VaD, and acknowledges the role of vascular risk factors in cognitive decline, such as type 2 diabetes mellitus (T2DM). Furthermore, VCI refers to different pathophysiological processes. One important underlying process is small vessel disease (SVD). Brain imaging, in particular magnetic resonance imaging (MRI), plays an important role in the assessment of these underlying processes.

This thesis focuses on brain imaging markers of T2DM as a risk factor for cognitive decline (**part I**), and on cerebral microvascular lesions as etiological and prognostic markers of VCI (**part II**).

Part I: Brain imaging markers in type 2 diabetes

T2DM is a risk factor for VCI. The exact underlying mechanisms of T2DM-associated cognitive dysfunction are, however, largely unknown. We used brain MRI to explore some of these mechanisms. In the first part of this thesis we examined structural and functional changes on brain MRI in T2DM. **Chapter 2** provides a review of the literature on brain imaging in T2DM. The main conclusion is that T2DM is associated with various forms of brain damage. The reported brain changes are heterogeneous, non-specific and only mildly distinct from changes occurring in normal aging. Most consistently, studies reported that people with T2DM show slightly more global brain atrophy than is seen in normal aging. Moreover, vascular lesions are seen more often, particularly lacunar infarcts. The association between T2DM and white matter hyperintensities and microbleeds is less clear. Brain imaging abnormalities in T2DM are also being studied using emerging imaging techniques, e.g. diffusion tensor imaging (DTI), functional MRI, and MR-spectroscopy. Such studies, however, are relatively scarce and thus far have not led to firm conclusions. The heterogeneity of brain imaging abnormalities that can be observed in patients with T2DM is reflected in the findings of chapters 3, 4 and 5.

In **chapter 3** we report on an imaging study measuring cortical surface, volume and thickness in 56 patients with T2DM and 30 controls. Total cortical surface, total cortical volumes and mean cortical thickness were consistently lower in the T2DM group (between group differences 0.5-4%). In particular, differences were found in the hippocampal region and in the middle temporal gyrus. In the T2DM group, smaller cortical thickness of the hippocampal region was associated with small vessel disease on MRI. This indicates that the effects of T2DM on the cortical gray matter are most pronounced in the temporal lobe, and this should probably be interpreted as a marker of mixed (i.e. degenerative and vascular) pathology.

In **chapter 4**, we explored both structural and functional brain MRI markers associated with T2DM. We examined how cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) are related to cognitive functioning and brain volumes, at baseline and after four years, in 114 people with T2DM. CBF, but not CVR, was associated with baseline cognitive performance and total brain volume. Neither CBF nor CVR predicted change in cognition of total brain volume after four years. These results do not support a causal role for disturbed haemodynamics in cognitive dysfunction and atrophy as might be inferred from the cross-sectional studies. Reverse causality might play a role, in other words, the demand for CBF may be relatively lower in abnormal atrophic brain tissue, thus explaining the baseline findings.

After investigating these conventional MRI markers, we assessed more subtle brain changes in people with T2DM at ultra-high field MRI. In **chapter 5**, we compared the occurrence of cortical microinfarcts and cerebral microbleeds at 7 Tesla MRI between 48 nondemented older individuals with T2DM and 49 control participants, and investigated the relationship with cognitive performance. We showed that microvascular lesions were common, but the occurrence did not significantly differ between patients (48%) and controls (38%). Furthermore, presence and number of microinfarcts or microbleeds were unrelated to cognitive performance. Since this is the first study on microvascular lesions at 7T MRI in patients with T2DM, and we assessed a relatively well-controlled patient group, further studies should assess whether cerebral microvascular lesions may occur in patients with T2DM who are less well controlled or have a high burden of microvascular complications elsewhere in the body.

To conclude, T2DM is associated with various changes on brain MRI, reflecting

neurodegenerative and vascular disease, both global and regional. These brain abnormalities are detectable at the group-level and are subtle as compared to normal ageing. Currently, diagnostic and prognostic values of individual MRI markers are insufficient with regard to T2DM-related cognitive decline. The challenge for further research will be to incorporate different imaging markers that add to the increased risk of dementia in a prediction model.

Part II: microvascular lesions

Conventional imaging markers of SVD (i.e. lacunar infarcts, white matter hyperintensities (WMH), brain atrophy) do not capture the full burden of cerebral microvascular damage. Autopsy studies point to the relevance of microinfarcts in the pathogenesis of cognitive decline and dementia. Microinfarcts are a common finding at autopsy, but evidence originates solely from post-mortem data and therefore the relation with age, vascular risk factors, and disease progression is largely unknown. At the start of my PhD project we set out to determine if ultra-high MR field strength enables visualization of microinfarcts *in vivo*.

Microbleeds also have attracted attention, due to their association with various clinical correlates including cognitive dysfunction and dementia. The number of microbleeds detected on MRI is highly dependent on the scan protocol and field strength. The possibility of 7T MRI to detect more microbleeds allows for further studies about their occurrence and their role in cognitive impairment.

In the second part of this thesis we examined microinfarcts and microbleeds as new markers of SVD. Firstly, we reviewed the available literature on neuropathological studies on cerebral microinfarcts, as a frame of reference for further MRI studies (**chapter 6**). The overall picture of these autopsy studies is that microinfarcts are more common in patients with dementia, particularly in patients with vascular dementia (62%) and Alzheimer's disease (43%), than in non-demented older individuals (24%). Microinfarcts are described as minute foci with neuronal loss, gliosis, pallor or more cystic lesions. Reported sizes vary from 50 μm to a few mm. These lesions may occur in all brain regions, although the cerebral cortex may be a preferential location. In the majority of cases multiple microinfarcts are observed. Based on these findings, we were able to develop scan protocols and criteria to detect cortical microinfarcts in vivo at ultra-high field MRI in different populations.

In **chapter 7**, we examined the occurrence of both microbleeds and microinfarcts - with a newly developed and validated protocol at 7T MRI - in a sample of

103 subjects without major neurological conditions, aged 40-80 years. We found that both microbleeds and cortical microinfarcts were present in a substantial proportion of the population. The number of microbleeds was related to age, but the number of microinfarcts was not. Neither the presence of microbleeds nor microinfarcts was related to vascular risk factors or other markers of SVD (lacunar infarcts, WMH volumes, total brain volume). We concluded that these lesions are common and already present in middle-aged persons, and that their occurrence is independent of other signs of SVD. Probably, the microvascular lesions we detected reflect pathophysiological processes that are different from other markers of SVD.

In **chapter 8** we showed that microbleeds at 7T MRI were present in the majority of the patients with mild cognitive impairment (MCI) or early stages of AD (78%), compared to 44% of controls. Microbleeds were unrelated to global cognitive performance. Prevalences were much higher than in studies using lower field strength. We concluded that presence of microbleeds may be the rule, rather than the exception in patients with MCI/AD. This fits with the assumption that microbleeds are the imaging correlate of cerebral amyloid angiopathy, which is a pathological process underlying AD.

Although microbleeds are related to modest cognitive deficits in the general population, this relationship in patients suffering from stroke is less clear. Therefore, we addressed this topic in chapter 9 and 10.

In **chapter 9**, we examined the relationship between microbleeds at conventional field strength in 328 patients with a TIA or minor ischemic stroke, and global cognitive performance four years later. We found that cognitive performance, measured with a telephone interview, did not differ between the 11.6% of patients with microbleeds and those without (relative risk for abnormal cognitive performance when having microbleeds: 1.19 (95% CI 0.63-2.26)).

In **chapter 10**, we investigated microbleeds at 7T MRI in a sample of patients with a TIA or ischemic stroke of the anterior circulation. The presence of microbleeds was higher than at regular field strength: 34%. In line with our results of chapter 9, we found no association between microbleeds and cognitive performance in four cognitive domains. Apparently, the association between a TIA or minor stroke and cognitive dysfunction is not mediated by the presence of microbleeds on MRI, contrary to other markers of small vessel disease.

To conclude, we are now, for the first time, able to visualize microinfarcts in vivo.

Detection however has to be optimized, and further studies are needed to elucidate associations between these detected microinfarcts on MRI and clinical correlates. The detection of microbleeds is highly increased at 7T MRI compared with regular field strength. Both microinfarcts and microbleeds at 7T MRI are common and present in healthy persons, but are not strongly related to other markers of SVD in these persons. On the other hand, microvascular lesions are more common in patients with stroke or dementia, but are not clearly related to cognitive performance in these patient groups. It appears that at the population-level, microvascular lesions could be a marker of future risk for stroke and cognitive decline. In patients with stroke or dementia, the overall burden of other vascular and neurodegenerative pathologies will be higher, which may mask the relatively subtle relation between microbleeds and cognition.

NEDERLANDSE SAMENVATTING

NEDERLANDSE SAMENVATTING

Dementie is een veel voorkomend probleem in onze vergrijzende samenleving. De meest voorkomende vormen van dementie zijn de ziekte van Alzheimer en vasculaire dementie. Het wordt steeds meer duidelijk dat vaatschade in het brein een belangrijke rol speelt in het ontstaan van dementie. Dit geldt niet alleen voor het ontstaan van vasculaire dementie, maar ook voor het ontstaan van de ziekte van Alzheimer. Daarom zou men beter kunnen spreken van een 'spectrum' van vormen van dementie, met aan de ene kant mensen waarbij de veranderingen in het brein puur door Alzheimer-pathologie veroorzaakt wordt, en aan de andere kant mensen met pure vaatschade in het brein. De meeste patiënten zullen echter een combinatie van beide vormen van breinschade hebben.

Vaatschade in het brein ontstaat niet alleen door bijvoorbeeld een beroerte, maar ook door de invloed van risicofactoren voor hart- en vaatziekten op het brein, zoals diabetes mellitus type 2 (suikerziekte), hoge bloeddruk, en overgewicht. Vaatschade is eigenlijk een heterogene term, waaronder bijvoorbeeld schade aan de grote en kleine bloedvaten valt, maar ook schade in het hersenweefsel zelf die het gevolg is van zieke bloedvaten. Het is nog onvoldoende duidelijk welke vormen van vaatschade in het brein leiden tot cognitieve beperkingen, en ook welke onderliggende processen bij welke mensen leiden tot vaatschade in het brein.

Ernstige cognitieve beperkingen door vaatschade zouden kunnen worden voorkomen, als deze vaatschade in een vroeg stadium wordt behandeld. Hiervoor is meer inzicht nodig in het beloop en in onderliggende processen van relevante vaatschade. Met behulp van magnetic resonance imaging (MRI) scans kunnen verschillende vormen van vaatschade onderzocht worden, evenals de samenhang met cognitief functioneren. Dit zal helpen om in de toekomst behandelstrategieën te ontwikkelen om de kans op cognitieve achteruitgang en dementie te verkleinen.

Deel 1 van dit proefschrift richt zich op verschillende markers van vaatschade in het brein bij mensen met diabetes mellitus type 2 (DM2), omdat deze patiënten een verhoogde kans hebben op het ontstaan van cognitieve beperkingen. De studies in **deel 2** richten zich op het voorkomen en de rol van 'microvasculaire laesies' (kleine bloedinkjes en infarctjes in de hersenen) in het ontstaan van cognitieve beperkingen.

Deel I: markers van schade in het brein bij diabetes mellitus type 2

Mensen met DM2 hebben een verhoogd risico op het ontwikkelen van cognitieve problemen en dementie. Het is nog niet volledig duidelijk welke processen in het brein hieraan bijdragen. MRI scans geven belangrijke informatie over deze

processen. **Hoofdstuk 2** geeft een overzicht van studies naar veranderingen in de hersenen bij patiënten met DM2. Deze studies laten zien dat DM2 geassocieerd is met verschillende vormen van breinschade, die heterogeen en niet-specifiek zijn, en bovendien niet veel verschillen van normale veroudering van het brein. Het meeste bewijs is er voor lichte afname van het breinvolume bij patiënten met DM2. Daarnaast worden vaker kleine herseninfarcten (lacunes) gezien. Het is minder duidelijk of patiënten met DM2 meer witte stofafwijkingen en microbloedingen hebben. Ten slotte zijn de laatste jaren nieuwe beeldvormende technieken ontwikkeld om naar subtiele schade in het brein te kijken, bijvoorbeeld functionele MRI. Er zijn echter nog te weinig studies verricht om duidelijke uitspraken te doen over bevindingen bij mensen met DM2. De heterogeniteit van veranderingen in het brein die in deze review beschreven is, komt ook naar voren in hoofdstuk 3, 4 en 5.

In **hoofdstuk 3** laten we zien dat de oppervlakte, het volume en de dikte van de hersenschors kleiner zijn bij de 56 patiënten met DM2 die wij vergeleken met controlepersonen. Deze verschillen bleken het meest uitgesproken te zijn in de schors van de mediale temporaalkwab. Dit specifieke hersengebied is ook aangedaan bij de ziekte van Alzheimer. In de diabetesgroep bleek een dunnere schors van de mediale temporaalkwab geassocieerd te zijn met markers van schade aan de kleine hersenvaatjes. Deze bevindingen suggereren dat het effect van DM2 op het breinvolume het meest uitgesproken is in de temporaalkwab, en dat een dunnere hersenschors in de temporaalkwab waarschijnlijk het gevolg is van zowel vaatschade als Alzheimer-pathologie.

Schade in het brein bij mensen met DM2 zou onder andere het gevolg kunnen zijn van veranderde haemodynamiek (bloeddoorstroming in de grote bloedvaten van de hersenen). Daarom hebben we in **hoofdstuk 4** patiënten met DM2 onderzocht, en gekeken naar de relatie tussen twee maten van bloedstroom naar de hersenen enerzijds, en breinvolumes en cognitief functioneren anderzijds op twee tijdspunten. Hieruit bleek dat een verminderde bloeddoorstroming geassocieerd was met kleinere breinvolumes en verminderd cognitief functioneren bij de eerste meting, maar dat dit niet gerelateerd was aan veranderingen in breinvolumes of cognitief functioneren na 4 jaar. Dit betekent dat er waarschijnlijk geen causaal verband is tussen de maten voor haemodynamiek en breinschade die wij onderzochten. Misschien is het wel andersom: abnormaal, verschrompeld hersenweefsel 'verbruikt' minder bloed.

Vervolgens hebben we gekeken naar subtiele veranderingen in het brein met behulp

van MRI scans op hoge veldsterkte. In **hoofdstuk 5** laten wij zien dat microbloedingen en microinfarcten niet vaker voorkomen bij mensen met DM2, en dat ze bovendien niet samenhangen met cognitief functioneren. Aangezien wij relatief 'goede' patiënten hebben onderzocht, met weinig DM2-gerelateerde complicaties, en omdat dit de eerste studie is naar microvasculaire laesies op hoge veldsterkte bij patiënten met DM2, zullen volgende studies moeten aantonen of deze laesies wel vaker voorkomen bij patiënten die meer vaatschade door DM2 hebben.

Conclusies

Zoals eerder gezegd, worden bij mensen met DM2 verschillende veranderingen in het brein gezien, zowel globaal als regionaal. Deze veranderingen zijn subtiel en worden met name op groepsniveau gezien. Voor de individuele patiënt hebben afzonderlijke MRI markers echter te weinig diagnostische en prognostische waarde voor cognitieve achteruitgang. Waarschijnlijk kunnen er pas uitspraken gedaan worden over de kans op cognitieve achteruitgang bij een individuele patiënt met DM2, wanneer verschillende MRI markers, waarvan bekend is dat ze samenhangen met cognitieve achteruitgang bij mensen met DM2, gecombineerd worden in één predictiemodel.

Deel II: microvasculaire laesies

Een belangrijke oorzaak van vaatschade in de hersenen vormt 'small vessel disease' (SVD). Hiermee bedoelen we zowel veranderingen in de kleine bloedvaatjes in het brein als de veranderingen in het hersenweefsel als gevolg hiervan. Veel onderzochte markers van SVD op MRI scans zijn lacunaire infarcten, witte stofafwijkingen en hersenatrofie. De laatste jaren is uit autopsie studies gebleken dat ook microinfarcten een belangrijke uiting zijn van SVD en dat zij gerelateerd zijn aan het voorkomen van dementie. Tot op heden waren microinfarcten alleen bij autopsie te onderzoeken, onder de microscoop. Met de komst van sterkere (7 Tesla) MRI-scanners is het sinds kort ook mogelijk deze laesies tijdens het leven af te beelden. Daarnaast geven deze 7 Tesla MRI scanners de mogelijkheid om microbloedingen beter af te beelden dan tot nu toe kon met MRI scanners met lagere veldsterkte. Microbloedingen zijn eveneens geassocieerd met cognitieve dysfunctie en dementie, en met de nieuwe beeldvormende technieken kunnen deze associaties nader onderzocht worden.

Omdat van microinfarcten tot kort geleden slechts bekend was hoe zij er onder de microscoop uitzagen, was het eerste doel om criteria te formuleren voor microinfarcten op een MRI scan. Dit hebben we gedaan door neuropathologische literatuur op

een rijtje te zetten (**hoofdstuk 6**). Deze studies lieten zien dat microinfarcten vaak voorkomen bij ouderen, met name bij mensen met de ziekte van Alzheimer of vasculaire dementie. De grootte varieert van 50 µm tot een paar mm. Ze kunnen overal in het brein gezien worden, meestal worden ook meerdere microinfarcten in één brein gezien, maar in de hersenschors lijken ze vaker voor te komen.

Vervolgens hebben wij het voorkomen van deze microvasculaire laesies (zowel microinfarcten als microbloedingen) op 7 Tesla MRI scans bekeken bij mensen zonder neurologische ziekte van 40-80 jaar oud (**hoofdstuk 7**). Uit deze studie bleek dat microvasculaire laesies bij een aanzienlijk gedeelte van de mensen gezien werden, niet alleen bij de ouderen. Het voorkomen van microbloedingen neemt toe met de leeftijd, het voorkomen van microinfarcten niet. Beide laesies zijn niet geassocieerd met andere tekenen van SVD op MRI-scans. Mogelijk weerspiegelen deze laesies andere ziekteprocessen aan de kleine bloedvaatjes dan de andere markers van SVD.

In hoofdstuk 8, 9 en 10 onderzochten wij microbloedingen bij verschillende patiënten: mensen met beginnende Alzheimer dementie en een beroerte.

In **hoofdstuk 8** laten we zien dat microbloedingen op 7 Tesla MRI-scans bij de meerderheid (78%) van de patiënten met vroege stadia van de ziekte van Alzheimer voorkomen, in tegenstelling tot eerdere MRI studies op lagere veldsterkte. Microbloedingen waren echter niet gerelateerd aan cognitief functioneren bij deze patiënten.

In **hoofdstuk 9 en 10** onderzochten we of microbloedingen bijdragen aan cognitieve dysfunctie die vaak optreedt na een TIA of herseninfarct. Wij vonden ook bij deze patiënten dat microbloedingen, zowel op lage veldsterkte (hoofdstuk 9) als hoge veldsterkte (hoofdstuk 10) niet gerelateerd waren aan cognitief functioneren. Blijkbaar komen microbloedingen vaak voor bij mensen met de ziekte van Alzheimer of een beroerte, maar hebben ze geen groot effect op cognitieve dysfunctie binnen deze patiëntengroepen.

Conclusies

Sinds kort maken sterkere 7 Tesla MRI scanners het mogelijk om microinfarcten tijdens het leven af te beelden. Detectie van microinfarcten zal verder geoptimaliseerd moeten worden en studies in de toekomst zullen meer kennis moeten geven over de associaties met klinische maten. Ook kunnen met deze 7 Tesla MRI scans microbloedingen beter afgebeeld worden dan met MRI scans op lagere veldsterkte. Uit de studies in dit proefschrift blijkt dat zowel microinfarcten als microbloedingen

vaak voorkomen, ook bij gezonde mensen, maar dat ze bij gezonde mensen niet sterk gerelateerd zijn aan andere markers van SVD. Aan de andere kant worden microvasculaire laesies vaker bij mensen met een beroerte en dementie gezien, maar zijn ze bij deze patiënten niet sterk gerelateerd aan cognitief functioneren. Het lijkt erop dat microvasculaire laesies bij jongere, gezonde mensen een proces kunnen vertegenwoordigen dat een verhoogd risico op een beroerte of dementie geeft. Patiënten die al een beroerte hebben doorgemaakt of dementie hebben, zullen ook (veel) andere schade in het brein hebben, waardoor het hebben van microbloedingen mogelijk slechts een kleine invloed heeft op cognitief functioneren.

ACKNOWLEDGEMENTS

DANKWOORD

LIST OF PUBLICATIONS

CURRICULUM VITAE

ACKNOWLEDGEMENTS

Chapter 2
The research of G.J.B. is supported by grant 2010T073 from the Dutch Heart Association and Vidi grant 91711384 from ZonMw, The Netherlands Organisation for Health Research and Development.

Chapter 3
This study was financially supported by grant 2003.01.004 of the Dutch Diabetes Research Foundation and by a high potential grant from Utrecht University.
The Utrecht Diabetic Encephalopathy Study Group consists of (in alphabetical order): A. Algra, E. van den Berg, G.J. Biessels, A.M.A. Brands, M.A. Breedijk, J. de Bresser, M. Brundel, J. van Gijn, W.H. Gispen, J. van der Grond, E.H.F. de Haan, A.C. van Huffelen, C. Jongen, L.J. Kappelle, R.P.C. Kessels, W.P.Th.M. Mali, S.M. Manschot, J.P.W. Pluim, Y.D. Reijmer, G.E.H.M. Rutten, A.M. Tiehuis, H.W. de Valk, M.A. Viergever.

Chapter 4
This study was financially supported by grant 2003.01.004 of the Dutch Diabetes Research Foundation and by a high potential grant from Utrecht University.
The Utrecht Diabetic Encephalopathy Study Group consists of (in alphabetical order): A. Algra, E. van den Berg, G.J. Biessels, A.M.A. Brands, M.A. Breedijk, J. de Bresser, M. Brundel, J. van Gijn, W.H. Gispen, J. van der Grond, E.H.F. de Haan, A.C. van Huffelen, C. Jongen, L.J. Kappelle, R.P.C. Kessels, W.P.Th.M. Mali, S.M. Manschot, J.P.W. Pluim, Y.D. Reijmer, G.E.H.M. Rutten, A.M. Tiehuis, H.W. de Valk, M.A. Viergever.

Chapter 5
The authors express their special thanks to the primary care practices Ametisthof, Glennhof, De Poort, 't Steyn, and De Weegbree of Huisartsenzorg IJsselstein, IJsselstein, the Netherlands (mentor Ph.L. Salomé), and G. Visser, Nieuwegein, the Netherlands, for their supportive role in the recruitment process.
This study was financially supported by Vidi Grant 91711384 from ZonMw, the Netherlands Organisation for Health Research and Development, and by Grant 2010T073 from the Netherlands Heart Foundation.

Chapter 6
The authors thank Professor Lon R. White (Pacific Health Research Institute, Kuakini Medical Center, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA) for his comments on a previous version of the manuscript. This study was financially supported by a high potential grant from Utrecht University.

Chapter 7
The authors express their special thanks to the primary care practices Ametisthof, Glennhof, De Poort, 't Steyn, and De Weegbree of Huisartsenzorg IJsselstein, IJsselstein, the Netherlands (mentor Ph.L. Salomé), and G. Visser, Nieuwegein, the Netherlands, for their supportive role in the recruitment process. The authors are grateful to the University Medical Center Utrecht Primary Care Network for participating in the PREDICT-MR study. We greatly acknowledge the use of MeVisLab by MeVis Medical Solutions AG, Bremen, Germany.
This study was financially supported by Vidi Grant 91711384 from ZonMw, the Netherlands Organisation for Health Research and Development, by Grant 2010T073 from the Netherlands Heart Foundation, and by a VIDI grant from the Netherlands Organisation for Scientific Research (NWO) (grant number 917-66-311).

Chapter 8
This study was funded by a high potential grant from Utrecht University and the "Internationale Stichting Alzheimer Onderzoek (ISAO)", grant 10506
The authors thank Hendrik de Leeuw (Image Sciences Institute, UMC Utrecht) for his work on the phase images.

Chapter 9
This work was supported by the Dutch Heart Association (grant 2001B071); the research of G.J.B. is supported by grant 2010T073 from the Dutch Heart Association. The authors thank mrs. Paut Greebe, mrs. Dorien C.V. Slabbers, mrs. Ans de Ridder, mrs. Saskia Scott, mrs. Olga A. Ternede, mrs. Moniek de Rijk, mrs. Sigrid van Steen, mrs. Jasmijn Y. Lodico- Kuipers, mrs. Sandra Kleijn, mrs. Hanneke M. Droste, mrs. Barbara F. Drijber, mrs. Helen Blad, mrs. Nadine Fleitour, mrs. Anick Gorissen, for collecting the data.
Participating centres MICRO (Microbleeds on MRI as predictors of IntraCerebral haemorrhage in patients Receiving Oral antithrombotic drugs after TIA or minor ischaemic stroke) Study Group (with numbers of patients included and

investigators): *University Medical Centre, Utrecht* (256, A. Algra, L.J. Kappelle, L.M.P. Ramos, E.L.L.M. De Schryver); *Slotervaart Hospital Amsterdam* (107, V.I.H. Kwa, G.J. Jöbsis, J.J. van der Sande); *Medisch Spectrum Twente, Enschede* (29, P.J.A.M. Brouwers); *Academic Medical Centre, Amsterdam* (17, Y.E.B.M. Roos, J. Stam); *Amphia Hospital, Breda* (15, S.L.M. Bakker, H.B.C. Verbiest); St. Antonius Hospital Nieuwegein (11, W.J. Schoonewille); *Central Military Hospital, Utrecht* (5, F.H.H.Linn); *Albert Schweitzer Hospital, Dordrecht* (3, L.I. Hertzberger); *Meander Medical Centre, Amersfoort* (3, H.M.A. van Gemert); *Lievensberg Hospital, Bergen op Zoom* (2, P.J.I.M. Berntsen).

Chapter 10

The research of GJB is supported by Vidi Grant 91711384 from ZonMw, the Netherlands Organization for Health Research and Development, and by Grant 2010T073 from the Netherlands Heart Foundation.

The research of JH is supported by Vidi Grant 016.126.322 from ZonMw, the Netherlands Organization for Health Research and Development

DANKWOORD

Dit proefschrift is tot stand gekomen met hulp en steun van vele anderen. Een aantal mensen wil ik graag ik het bijzonder noemen.

In de eerste plaats gaat mijn dank uit naar alle **deelnemers** die de afgelopen jaren vrijwillig hebben deelgenomen aan de Utrecht Diabetes Encephalopathy Study ($UDES_2$) en de Intracranial Vessel wall Imaging (IVI) studie.

Prof. dr. G.J. Biessels, beste Geert Jan, jouw enthousiasme voor je onderzoeksveld in combinatie met onderzoek doen op het grensvlak van neurologie, radiologie en neuropsychologie, heeft mij over de streep getrokken dit onderzoek aan te gaan. Stap voor stap heb je mij alle facetten van de wetenschap geleerd, van SPSS tot het bijhouden van een logboek. Ik heb grote bewondering voor de manier waarop je 'grote lijnen' kunt zien, discussies concreet kunt maken, en voor je pragmatische instelling, ook in de kliniek. Daarnaast wist je me elke keer weer op te beuren als de moed mij in de schoenen zonk omdat een project niet precies ging zoals ik had bedacht. Ten slotte complimenten voor de hechte onderzoeksgroep, zowel op als buiten het werk, die je hebt weten te vormen!

Prof. dr. L.J. Kappelle, beste Jaap, jouw optimisme en gave om de essentie te zien waren heel waardevol bij de verschillende projecten. Jouw advies om de rust te nemen om nog één keer kritisch naar een stuk te kijken (niet mijn tweede natuur), bleek elke keer weer te helpen. Veel dank ook voor het meegaan in mijn ambitieuze planning deze laatste maanden.

De leden van de beoordelingscommissie, **Prof. dr. Ir. M.A. Viergever**, **Prof. dr. A. Algra**, **Prof. dr. L.H. van den Berg**, **Prof. Dr. H.C. Dijkerman** en **Prof. dr. M. Olde Rikkert**, dank ik voor hun bereidheid dit manuscript te lezen en te beoordelen.

Mede-auteurs van de hoofdstukken uit dit proefschrift, **dr. H.L. Koek**, **dr. V.I.H. Kwa**, **Prof. dr. A. Algra**, **dr. J. Hendrikse**, **Prof. dr. P.R. Luijten**, **dr. M.I. Geerlings**, **Jeroen van Dillen**, dank voor de samenwerking. Beste Vincent, hartelijk dank voor de mogelijkheid om aan te haken bij de MICRO studie.

Prof. dr. J.H.J. Wokke, graag wil ik u bedanken voor de mogelijkheid de opleiding tot

neuroloog in Utrecht te doorlopen, en om deze te combineren met wetenschappelijk onderzoek.

Dr. C.J.M. Klijn, beste Karin, ook al was je bij dit proefschrift niet betrokken, jij bent wel degene geweest die mij kennis heeft laten maken met en enthousiast wist te maken voor de wetenschap.
Kim, ik zal de periode in het lab en de eindeloze uren in de auto het land door (onder het genot van zakken chips) om bloed te prikken bij de AVM-patiënten, niet snel vergeten. Ik ben blij dat ik je toen heb leren kennen, niet alleen op maar ook buiten het werk!

Martijn van den Heuvel, samen hebben we in een record tempo mijn eerste publicatie een feit gemaakt. Dank voor je enthousiasme, kritische vragen en voor de samenwerking.

Kamergenoten van kamertje 1: **Monique**, **Marcel**, **Eduard**, **Janneke**, **Merel**, **Rachel**, **Sophie**, **Yaël**, **Willem**, **Susanne**, **Lieza** en **Onno**. Kamertje 1 voelde bijna als een tweede thuis, en onderzoek doen was absoluut een stuk leuker dankzij jullie!

Yaël, je hebt me denk ik meer over onderzoek geleerd dan je je realiseert. Na de METC-ellende hebben we de UDES2 samen tot een goed einde gebracht, en het was niet alleen bijzonder productief samenwerken, maar ook heel gezellig!

Sophie, dank voor de samenwerking bij de Parelsnoer-projecten, de Excel-formules, je enthousiasme voor het vieren van feestdagen met versiering en ontbijtjes, en voor de briljante avonden (nachten) in de kroeg.

Susanne, het was de bedoeling dat ik jou zou begeleiden in het microinfarcten-project. Al snel was het precies andersom. Het is heel leuk en aanstekelijk om te zien hoe enthousiast je wordt van deze kleine laesies.

Willem, dank voor de honderden scans die we samen hebben gescoord (met meer en minder moderne technieken), en excuses voor mijn strakke deadlines…

Alle overige leden van de **VCI groep**, in het bijzonder Jeroen, Laura, Minke en Erika, dank voor de samenwerking bij verschillende projecten en de gezelligheid tijdens congressen!

Hugo, zonder jou was dit proefschrift zeker weten nog niet af geweest. Als ik uren bezig was geweest met het omzetten van data, kreeg jij het op de een of andere manier in een paar minuten voor elkaar. Dank voor al je hulp, geduld, inventieve oplossingen en voor de samenwerking bij verschillende artikelen!

Anja, ik kon aanhaken bij de IVI studie en samen hebben wij het scannen van klinische patiënten op de 7 Tesla MRI scanner opgezet. Dit ging niet altijd van een leien dakje. Jouw enthousiasme en doorzettingsvermogen maakten echter dat het leuk bleef. Ik hoop dat we nog veel chocomelletjes samen zullen drinken!

Alle andere mensen van de radiologie en de 7T groep die dit onderzoek mede mogelijk gemaakt hebben, in het bijzonder **Fredy Visser, Jaco Zwanenburg** en alle **MRI laboranten**, veel dank voor alle hulp, de fijne samenwerking en de gezelligheid bij de scanner.

Collega's van de neurologie, jullie maken dat werk vaak helemaal niet als werk voelt. Dr. D en dr. B, bedankt voor het lachen! Marcel, Christiaan, Perry en Oliver, de assistentenweekenden vorm(d)en natuurlijk het hoogtepunt van het jaar.

Lieve **Nora**, we leerden elkaar via Unitas kennen, en hoe gezellig dat we nu samen de opleiding in Utrecht doen! Maar, veel belangrijker, je bent de beste roomie voor elk (neuro-)uitje en een top vriendin. Het voelt niet meer dan logisch dat je op 2 september achter mij staat.

Evelien en Bart, andere twee MDT-ers, dank voor alle gezelligheid de afgelopen jaren!

Lieve **Marieke**, wat een toptijd hadden we op de Asterstraat (ondanks bepaalde huisgenoten), en wat hebben we sindsdien veel samen meegemaakt! Helaas woon je niet meer om de hoek, maar dat maakt niet dat je minder dichtbij voelt. Dank voor alles.

Lieve **Annegreet**, na een heerlijke periode op Curaçao was het voor ons allebei tijd om hard te werken. Met niemand kan ik beter klagen maar vooral ook lachen om promotieperikelen en om alle andere belangrijke dingen in het leven. Laten we onze 'werklunches' er vooral inhouden!

Lieve **Anna**, **Marijn**, **Valérie**, **Go** en **Annelotte**, wat hebben we in de ruim elf jaar dat we elkaar nu kennen veel samen meegemaakt! De wekelijkse etentjes, feestjes, weekendjes en vakanties hebben mijn studententijd absoluut leuker gemaakt en mij vervolgens ook door mijn promotietraject heen geholpen. Ik kijk uit naar alle leuke dingen die we samen nog gaan doen, met en zonder kinderen!

Lieve **Lena**, **Roos**, **Annelien**, **Anouk** en **Elianne**, omdat we elkaar al zo lang kennen hebben we aan een half woord genoeg. De schooljaren en vele vakanties hebben plaats gemaakt voor iets minder frequente maar zeker niet minder gezellige dates. Het is heel waardevol om een groep vriendinnen te hebben die je kennen vanaf je tuinbroeken-tijd.

Beste **familie Ton**, Fred, Corrie, Erik, Rianne, Amber en Pepijn, wat is het fijn om zo'n gezellige schoonfamilie dichtbij ons te hebben. Dank voor alle (praktische) hulp en steun de afgelopen jaren.

Lieve **mam** en **Vincent**, **pap** en **Wendela**, **Jeroen** en **Manon**. Familie is de basis, en zo voelt het ook. Pap en mam, jullie hebben me gemaakt tot wie ik nu ben, en geven de liefde en steun die ik iedereen zou wensen. Broeder, dank voor je interesse in mijn onderzoek en onze discussies over (voor mij meer en minder begrijpelijke) natuurkundige zaken.
Tijmen, kleine grote broer. Het zou me niet verbazen als jij ooit ook onderzoek gaat doen. Het is heerlijk om te zien hoe dol je op Féline en Nora bent, je moet maar vaak bij ons komen logeren!

Liefste **Marcel**, wat ben ik blij met jou. Je hebt vaak niet door hoeveel jouw nuchtere blik en relativerende woorden mij hebben geholpen als ik weer eens vond dat het niet goed genoeg was of niet snel genoeg ging. Je helpt mij om los te laten en te lachen, ook als ons leven eigenlijk weer iets te druk is. Samen lukt het ons.

Féline en **Nora**, mijn twee lieve aapjes, wat maken jullie mij gelukkig. In mijn buik hebben jullie al heel wat uren achter de computer doorgebracht terwijl ik met dit onderzoek bezig was. Heerlijk dat ik mijn proefschrift af heb nu jullie er allebei zijn, ik verheug me er enorm op om samen heel veel leuke dingen te gaan doen!

LIST OF PUBLICATIONS

In this thesis

Brundel M, Reijmer YD, van Veluw SJ, Kuijf HJ, Luijten PR, Kappelle LJ, Biessels GJ; on behalf of the Utrecht Vascular Cognitive Impairment (VCI) Study Group. Cerebral microvascular lesions on High-Resolution 7T MRI in patients with type 2 diabetes. *Diabetes* 2014 [Epub ahead of print]

Brundel M, Kappelle LJ, Biessels GJ. Brain imaging in type 2 diabetes. *Eur Neuropsychopharmacology* 2014 [Epub ahead of print]

Brundel M, Kwa VIH, Bouvy WH, Algra A, Kappelle LJ, Biessels GJ; on behalf of the MICRO Study Group. Cerebral microbleeds are not associated with long-term cognitive outcome in patients with transient ischemic attack or minor stroke. *Cerebrovas Dis* 2014;37:195-202.

Brundel M, Heringa SM, de Bresser J, Koek HJ, Zwanenburg JJM, Kappelle LJ, Luijten PL, Biessels GJ. High prevalence of cerebral microbleeds at 7Tesla MRI in patients with early Alzheimer's Disease. *J. Alzheimers Dis*. 2012;31:259-63.

Brundel M, van den Berg E, Reijmer YD, de Bresser J, Kappelle LJ, Biessels GJ; On behalf of the Utrecht Diabetic Encephalopathy Study Group. Cerebral haemodynamics, cognition and brain volumes in patients with type 2 diabetes. *J Diabetes Complications*. 2012;26:205-9.

Brundel M, de Bresser J, van Dillen J, Kappelle LJ, Biessels GJ. Cerebral microinfarcts: a systematic review of neuropathological studies. *J Cereb Blood Flow Metab*. 2012;32:425-36.

Brundel M, van den Heuvel M, de Bresser J, Kappelle LJ, Biessels GJ; On behalf of the Utrecht Diabetic Encephalopathy Study group. Cerebral cortical thickness in patients with type 2 diabetes. *J Neurol Sci*. 2010;299:126-30.

Other publications

Wisse LEM, de Bresser J, Geerlings MI, Reijmer YD, Portegies MP, **Brundel M**, Kappelle LJ, van der Graaf Y, Biessels GJ, on behalf of the Utrecht Diabetic Encephalopathy Study Group and the SMART-MR study group. Global brain atrophy but not hippocampal atrophy is related to type 2 diabetes. *J Neurol Sci*. 2014 [Epub ahead of print].

Brundel M*, Boshuisen K*, de Kovel CGF, Letteboer TG, Rinkel GJE, Westermann CJJ, Koeleman BPC, Klijn CJM. Polymorphisms in the ACVRL1 and endoglin genes in sporadic brain arteriovenous malformations and in vascular malformations in the brain associated with hereditary haemorrhagic telangiectasia. *Translational stroke research* 2013;4:375-78.

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

Kwa VIH, Algra A, **Brundel M**, Bouvy W, Kappelle LJ, on behalf of the MICRO Study Group. Microbleeds as a predictor of intracerebral haemorrhage and ischaemic stroke after a TIA or minor ischaemic stroke: a cohort study. *BMJ Open* 2013;3: e002575.

Van der Kolk AG, Hendrikse J, **Brundel M**, Biessels GJ, Smit EJ, Visser F, Luijten PR, Zwanenburg JJ. Multi-sequence whole-brain intracranial vessel wall imaging at 7.0 tesla. *Eur Radiol* 2013;23:2996-3004.

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: 2112-15.

Reijmer YD, **Brundel M**, de Bresser J, Kappelle LJ, Leemans A, Biessels GJ; Utrecht Vascular Cognitive Impairment Study Group. Microstructural white matter abnormalities and cognitive functioning in type 2 diabetes: a diffusion tensor imaging study. *Diabetes Care*. 2013;36:137-44.

De Bresser J, **Brundel M**, Conijn MM, van Dillen JJ, Geerlings MI, Viergever MA, Luijten PR, Biessels GJ. Visual cerebral microbleed detection on 7T MRI: reliability and effects of image processing. *AJNR Am J Neuroradiol* 2013;34:E61-4.

Brundel M, Kappelle LJ, Biessels GJ. Cerebrale micro-infarcten. *Neuropraxis* 2012;16:173-182.

Van der Kolk AG, Zwanenburg JJM, **Brundel M**, Biessels GJ, Visser F, Luijten PR, Hendrikse J. Intracranial vessel wall imaging at 7.0 Tesla MRI. *Stroke* 2011;42:2478-84.

Kloppenborg RP, Zwanenburg JJM, Luijten PR, **Brundel M**, Hendrikse J, Nederkoorn PJ. Beeldvorming van kleine bloedvaten in de hersenen met 7 Tesla MRI. *NTvG* 2011;155:A3232.

*These authors contributed equally to the manuscript

CURRICULUM VITAE

Manon Brundel werd geboren op 15 februari 1984 te 's-Gravenhage. In 2002 behaalde zij haar gymnasiumdiploma aan het Stedelijk Gymnasium Johan van Oldenbarnevelt te Amersfoort. Aansluitend begon zij aan haar studie geneeskunde aan de Universiteit Utrecht. Haar interesse in de neurologie werd groter naarmate de studie vorderde, waarop zij in het laatste jaar een wetenschappelijke stage op de afdeling neurologie van het Universitair Medisch Centrum Utrecht volgde, onder begeleiding van dr. C.J.M. Klijn en prof. dr. G.J.E. Rinkel. Dit project naar genetische achtergronden van cerebrale arterioveneuze malformaties heeft geleid tot een wetenschappelijke publicatie. In 2008 sloot zij haar studie af met een semi-artsstage neurologie in het Universitair Medisch Centrum Utrecht en een co-schap psychiatrie in Klinika Capriles op Curaçao. Na het behalen van haar artsexamen startte zij als arts-assistent neurologie in het Universitair Medisch Centrum Utrecht. In 2009 begon zij hier met de opleiding tot neuroloog onder begeleiding van prof. dr. J.H.J. Wokke. In datzelfde jaar begon zij aan haar promotieonderzoek onder begeleiding van prof. dr. G.J. Biessels en prof. dr. L.J. Kappelle, wat heeft geleid tot dit proefschrift. Zij verwacht haar opleiding tot neuroloog af te ronden in 2019.